Appendix A

Workshop Agenda

Perchlorate Peer Review Workshop
Agenda
San Bernardino City Council Chambers
San Bernardino, California
February 10 - 11, 1999

Wednesday, February 10

8:30 - 8:35	Opening Remarks - Susan Goldhaber, Research Triangle Institute (RTI)
8:35 - 8:40	Opening Remarks - Dr. Curtis Klaassen, Workshop Chair
8:40 - 8:45	Background - Peter Grevatt, EPA Office of Solid Waste and Emergency Response (OSWER)
8:45 - 8:55	Background - Kevin Mayer, EPA Region IX
8:55 - 10:10	Presentation: Toxicological Review/Reference Dose/Cancer Assessment Policy- Dr. William Farland, Annie Jarabek, EPA Office of Research and Development (ORD)
10:10 - 10:25	BREAK
10:25 - 12:00	Presentations from Observers
12:00 - 1:00	LUNCH
1:00 - 3:30	Discussion of Toxicity Database and Draft Toxicological Review Document - Dr. David Brusick, Facilitator/Peer Reviewer General Statistical Issues - Dr. Haseman 90-Day Subchronic Oral Bioassay Study - Dr. Porterfield/Dr. Emerson Neurobehavioral Developmental Study - Dr. Zoeller/Dr. Emerson Pilot Developmental Study/Segment II Developmental Study - Dr. Tyl 2-Generation Reproductive Study - Dr. Tyl Immunotoxicity Studies - Dr. White/Dr. Zoeller Genotoxicity Studies - Dr. Brusick Ecotoxicity Studies - Dr. Cardwell
3:30 - 3:45	BREAK
3:45 - 6:00	Continued Discussion of Toxicity Database and Draft Toxicological Review Document

Perchlorate Peer Review Workshop
Agenda
San Bernardino City Council Chambers
San Bernardino, California
February 10 - 11, 1999

Thursday, February, 11

8:00 - 10:00	Continued Discussion of Draft Toxicological Review Document/ Hazard Characterization - Dr. David Brusick, Dr. Mel Andersen, Facilitators/Peer Reviewers
10:00 - 10:15	BREAK
10:15 - 12:00	Continued Discussion of Hazard Characterization/Additional Studies Required - Dr. Andersen, Facilitator/Peer Reviewer
12:00 - 12:30	Workshop Summary - Dr. Klaassen, Workshop Chair

Appendix B

List of Peer Reviewers

Peer Reviewers for Perchlorate Workshop

Chair

Dr. Curtis Klaassen
University of Kansas Medical Center
2018 Breidenthal Building
3901 Rainbow Boulevard
Kansas City, KS 66160
(913) 588-7714

Peer Reviewers

Dr. Melvin Andersen
Colorado State University
Center for Environmental Toxicology and Technology
Fort Collins, CO 80523-1680
(970) 491-8522

Dr. David Brusick
Covance Laboratories, Inc.
9200 Leesburg Pike
Vienna, VA 22182
(703) 893-5400

Dr. Rick Cardwell
Parametrix, Inc.
5808 Lake Washington Blvd. N.E. Suite 200
Kirkland, WA 98033-7350
(425) 822-8880

Dr. Charles Emerson
University of Massachusetts Medical Center
55 Lake Avenue North
Worcester, MA 01655
(508) 856-3166

Dr. Joseph Haseman
National Institute of Environmental Health Sciences
Biostatistics Branch
P.O. Box 12233
Research Triangle Park, NC 27709
(919) 541-4996

Dr. Susan Porterfield
Medical College of Georgia
CB-1104
Augusta, GA 30912-4765
(706) 721-3217

Dr. Rochelle Tyl
Research Triangle Institute
Center for Life Sciences and Toxicology
P.O.Box 12194
Research Triangle Park, NC 27709
(919) 541-5972

Dr. Kimber White
Medical College of Virginia
527 North 12th Street
Strauss Immunotoxicology Research Laboratory
Room 2011
Richmond, VA 23298
(804) 828-6789

Dr. R. Thomas Zoeller
University of Massachusetts
Department of Biology
Morrill Science Center
Amherst, MA 01003
(413) 545-2088

Appendix C

Short Resumes of Peer Reviewers

CURRICULUM VITAE

Curtis Dean Klaassen, Ph.D.

PLACE AND DATE OF BIRTH:

Fort Dodge, Iowa
November 23, 1942

[redacted]

DEGREES:

1964 B.A. - Wartburg College (Biology)
1966 M.S. - University of Iowa (Pharmacology)
1968 Ph.D. - University of Iowa (Pharmacology)

CERTIFICATION:

1980 American Board of Toxicology
1992 The Academy of Toxicological Sciences (Honorary member)

ACADEMIC APPOINTMENTS:

1968-1970	Instructor of Pharmacology and Toxicology, University of Kansas Medical Center
1970-1974	Assistant Professor of Pharmacology and Toxicology, University of Kansas Medical Center
1974-1977	Associate Professor of Pharmacology and Toxicology, University of Kansas Medical Center.
1975	Guest Professor of Clinical Pharmacology, University of Bern, Bern, Switzerland, June-August
1977-pres	Head, Section on Toxicology, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center
1977-pres	Professor of Pharmacology and Toxicology, University of Kansas Medical Center
1978	Visiting Scientist, Department of Toxicology, Institute of Radiation and Environmental Research (GSF), Munich, Germany, March-August
1984-pres	Professor of Molecular Cytology, Institute of Investigative Cytology, Valencia, Spain
1986-1989	Associate Director, Environmental Health and Occupational Medicine Center, University of Kansas Medical Center
1989-1991	Interim Director, Environmental Health and Occupational Medicine Center, University of Kansas Medical Center

HONORS:

1. 1964 Magna Cum Laude, Wartburg College
2. 1965-1968 Public Health Service Predoctoral Fellowship Award, NIH

3. 1971-1976 Public Health Service Research Career Development Award, NIH
4. 1976 Achievement Award, Society of Toxicology
5. 1978 Alexander von Humboldt Fellow
6. 1982-1987 Burroughs Wellcome Scholar in Toxicology
7. 1982-1983 MASUA Honor Lecturer
8. 1982 KUMC Research Award
9. 1985 Distinguished Visiting Professor, New Mexico State University
10. 1985 KU-Higuchi Research Award (The Dolph Simons, Sr. Research Award)
11. 1986 Wartburg College Alumni Citation
12. 1987 Distinguished Visiting Professor, University of Toledo
13. 1993 Eugene Garfield in *Current Contents* (January 18, 1993) indicated that between 1980 and 1992, Curtis Klaassen:
    A. Published 115 peer-reviewed scientific publications on the study of xenobiotics.
    B. Had the 12th highest scientific impact (2,227 references) in the world in the study of xenobiotics.
    C. Had the 4th highest scientific impact in the United States in the study of xenobiotics.
    D. Had the greatest scientific impact in the world in the area of toxicology.
14. 1993 Educational Award, Society of Toxicology
15. 1993 Chancellor's Club Research Award, University of Kansas
16. 1994 Kenneth P. DuBois Award, by Midwest Regional Chapter of the Society of Toxicology
17. 1994 William P. Kinter Memorial Lectureship, Mount Desert Island Biological Laboratory, Maine
18. 1998 Founders Award, CIIT
19. 1998 John Doull Award, Central States Chapter of the Society of Toxicology

PROFESSIONAL SOCIETIES:

1. 1968 Sigma Xi
2. 1969 American Association for the Advancement of Science
3. 1970 Society of Toxicology
4. 1970 American Society of Pharmacology and Experimental Therapeutics
5. 1971 American Association for the Study of Liver Diseases
6. 1972 Society of Experimental Biology and Medicine
7. 1993 International Society for the Study of Xenobiotics (ISSX)
8. 1998 Founders Award, CIIT
9. 1998 John Doull Award, Central States Chapter of the Society of Toxicology

EDITORIAL BOARDS:

1. 1974-1998 *Journal of Pharmacology and Experimental Therapeutics*, Toxicology Field Editor
2. 1976-1978 *Chemico-Biological Interactions*, Editorial Board
3. 1977-pres *Journal of Pharmacological and Toxicological Methods*, Associate Editor
4. 1980-1990 *Toxicology and Applied Pharmacology*, Associate Editor
5. 1980-1983 *Hepatology, Editorial Board*

6. 1980-pres *Journal of Toxicology and Environmental Health*, Editorial Board
7. 1984-1993 *Xenobiotica*, Editorial Board
8. 1988-1989 *ISI Atlas of Science*: Pharmacology Advisory Editor
9. 1992-1998 *Chemico-Biological Interactions*, Editorial Board
10. 1993-1996 *Regulatory Toxicology and Pharmacology*, Editorial Board
11. 1996-pres *Current Protocols in Pharmacology*, Editorial Board
12. 1997-pres *Toxicological Sciences*, Editor-in-Chief

NATIONAL and INTERNATIONAL COMMITTEES:

Elected:

Society of Pharmacology and Experimental Therapeutics

1. 1976-1979 Executive Committee of the Drug Metabolism Division, American Society of Pharmacology and Experimental Therapeutics
2. 1977-1979 Treasurer of the Drug Metabolism Division, American Society of Pharmacology and Experimental Therapeutics

Society of Toxicology

1. 1979-1981 Education Committee, Society of Toxicology, Chairman 1980-1981
2. 1981-1984 Membership Committee, Society of Toxicology, Chairman 1983-1984
3. 1983-1986 Councilor, Mechanism Subsection, Society of Toxicology
4. 1983-1985 Councilor, Metals Subsection, Society of Toxicology
5. 1985-1987 Councilor, Society of Toxicology
6. 1988-1989 Vice-President Elect, Society of Toxicology
7. 1988-1990 Program Committee, Society of Toxicology, Chairman 1989-90
8. 1989-1990 Vice-President, Society of Toxicology
9. 1989-1991 Board of Publications, Society of Toxicology
10. 1989-1991 Finance Committee, Society of Toxicology
11. 1990-1991 President, Society of Toxicology
12. 1991-1992 Past-President, Society of Toxicology
13. 1991-1992 Awards Committee, Society of Toxicology, Chairman
14. 1991-1992 Ethics Committee, Society of Toxicology, Chairman
15. 1989-1993 Toxicology Education Foundation Board of Trustees, Vice President 1991-92, President 1992-93
16. 1992-1993 Nominating Committee, Society of Toxicology, Chairman
17. 1994-1995 Nominating Committee, Society of Toxicology
18. 1998-present Nominating Committee, Society of Toxicology

International Union of Toxicology

1. 1989-1992 Director, International Union of Toxicology (IUTOX)
2. 1992-1995 President, International Union of Toxicology (IUTOX)
3. 1995- Past President, International Union of Toxicology (IUTOX)

International Society of the Study of Xenobiotics

1. 1997- Councilor

Wartburg College

1. 1992-1995 Wartburg College Alumni Board

MELVIN ERNEST ANDERSEN, Ph.D., DABT, CIH

Professor, Environmental Health
Colorado State University
Fort Collins, CO 80523

Business Address
Colorado State University
Fort Collins, CO 80523
(970) 491-8522
(970) 491-8304 Fax
andersenme@aol.com

[redacted]

Dr. Andersen joined the faculty of Colorado State University in January 1999. He serves as Professor of Environmental Health and Director of the University's International Center for Risk Assessment, an organization that emphasizes novel approaches for applying emerging scientific knowledge to improve environmental and human health risk assessment and risk management. From 1994-1998, Dr. Andersen was Vice-President of the K.S. Crump Group of ICF Kaiser International Consulting. Between 1971 and 1994, he held positions in toxicology research and research management in the federal government (DoD and US EPA) and in private industry (Chemical Industry Institute of Toxicology). His career contributions are mainly in developing biologically realistic models of the uptake, distribution, metabolism, and biological effects of toxic chemicals and drugs and applying these models in safety assessments and quantitative health risk assessments. He is widely known for developing short-courses and computer demonstrations in pharmacokinetics and pharmacodynamic modeling. Dr Andersen is an author on over 180 papers and 30 book chapters. He has received many awards for professional contributions. These awards include the Stokinger Award (American Conference of Governmental Industrial Hygienists, 1988), the George Scott Award (Toxicology Forum, 1993), the Kenneth Morgareidge Award (International Life Sciences Institute, 1989), and the Frank R. Blood (1982) and Achievement Awards (1984) from the Society of Toxicology.

EDUCATION:

1971 Ph.D., Biochemistry and Molecular Biology, Cornell University, Ithaca, NY
1967 Sc.B., Chemistry, Brown University, Providence, RI

BOARD CERTIFICATIONS:

Certified in the Comprehensive Practice of Industrial Hygiene (1978). Certificate No. 1331.
Diplomate of the American Board of Toxicology (1981).

SOCIETY MEMBERSHIPS:

- Society of Toxicology
- American Academy of Industrial Hygiene
- American Conference of Governmental Industrial Hygienists; Associate Member
- Risk Assessment Section, Society of Toxicology
- Society of Risk Analysis

AWARDS:

George H. Scott Award of the Toxicology Forum for significant original contributions to the science of toxicology, 1993.

Best Industrial Hygiene related paper appearing in AIHA Journal or Applied Industrial Hygiene in 1989. Presented by the Michigan Society of Industrial Hygiene.

The Kenneth Morgareidge Award for Outstanding Research Contributions to the Science of Inhalation Toxicology, International Life Sciences Institute (ILSI), 1989.

Herbert Stokinger Award for Outstanding Contributions in Industrial Toxicology, American Industrial Hygiene Association, 1988.

Achievement Award, Society of Toxicology for Outstanding Contributions in Toxicology by a scientist less than 41 years of age. 1984

Paper of the Year, Inhalation Specialty Section, Society of Toxicology, 1985.

Award for Outstanding Professional Achievement, Affiliate Societies Council, Engineering and Science Foundation of Dayton, 1985.

Frank R. Blood Award - Society of Toxicology - 1982 (best paper in Toxicol. Appl. Pharmacol. for period 6/1980-5/1981).

Selection as an 'International Man of the Year', International Biographical Centre, Cambridge, England, 1992-1993.

Harry G. Armstrong Award for Scientific Excellence, AFAMRL, 1982.

DuPont Award, for excellence in teaching biochemistry; Cornell University, 1970.

Francis Wayland Scholar, Brown University, 1967.

Listed in American Men & Women of Science, 1995.

Listed in Who's Who in Science and Engineering, 1996.

Listed in Who's Who in America. 51st Edition 1997.

EDITORIAL BOARDS:

Member, Editorial Board, Food and Chemical Toxicology, 1991-1996.

Member, Editorial Board, Toxicology and Applied Pharmacology, 1985-1995.

Member, Editorial Board, Inhalation Toxicology, 1988-Present.

Member, Editorial Board, Human and Experimental Toxicology, 1994-Present.

Member, Editorial Board, Human and Ecological Risk Assessment, 1994-Present.

Associate Editor, Toxicology and Applied Pharmacology, 1982-1984.

CURRENT FACULTY APPOINTMENTS::

Adjunct Professor, Louisiana Tech University, Ruston, LA. 1996-2001.

Adjunct Professor in Medicine, Duke University, Duke University Medical Center, Durham, NC, 1 May 1994 - 30 June 1995

WORK EXPERIENCE:

1994-1999	The K.S. Crump Group, ICF Kaiser Consulting, Research Triangle Park, NC 27709, Vice-President
1993-1994	US Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC (Senior Scientist)
1992-1993	Duke University School of Medicine (Research Professor).
1989-1992	Chemical Industry Institute of Toxicology, Research Triangle Park, NC (Department Head, Risk Assessment and Senior Scientist)

1978-1989 Civil Service, Department of Defense, Toxic Hazards Division, Wright- Patterson AFB, Ohio (Director)

1971-1978 Active Duty, US Navy, Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson AFB, Ohio (Department Head)

OTHER POSITIONS:

Member, Committee on Toxicology, National Research Council, 1999-2000.

Chairperson, Expert Panel Evaluating EPA's Guidelines for Carcinogen Risk Assessment Using Chloroform and Dichloroacetate as Case Studies. ILSI, 1996-1997.

Co-Chairperson, Society of Toxicology, Task Force to Improve the Scientific Basis of Risk Assessment, 1996-1998.

American Board of Toxicology, 1991-1995.

American Conference of Governmental Industrial Hygienists (ACGIH) ACGIH Liaison to AIHA Unusual Workshift Committee, 1983-1984.

Threshold Limit Value Airborne Contaminants Committee, 1981-1984.

Safe Drinking Water Committee, National Academy of Sciences, Washington, D.C., 1984-1985.

Education Committee, Society of Toxicology, 1983-1985.

Awards Committee, Society of Toxicology, 1987-1989.

Scientific Advisory Panel, Chemical Industry Institute of Toxicology, 1986-1988.

Member, Pharmacokinetic Subcommittee, Safe Drinking Water Committee, National Academy of Sciences, 1986-1987.

DAVID J. BRUSICK, Ph.D., A.T.S.

VICE PRESIDENT, MAMMALIAN TOXICOLOGY
COVANCE LABORATORIES INC.
NORTH AMERICA

EDUCATION

NAS/NRC Postdoctoral Fellow 1970 -1971.

Ph.D., Microbial Genetics, Illinois State University, Normal, Illinois, 1970.

M.S., Genetics, Illinois State University, Normal, Illinois, 1965.

B.S., Biology, University of Illinois, Urbana, Illinois, 1963.

BACKGROUND

1997 - Present	Vice President, Mammalian Toxicology, Covance Laboratories Inc., Vienna, Virginia.
1995 -1997	Director CHNA Toxicology
1988 - 1995	Director, Corning Hazleton North America Toxicology, Corning Hazleton Inc., Vienna, Virginia.
1986 - 1987	Director, Molecular Toxicology Division, Hazleton Laboratories America, Inc., Kensington, Maryland.
1985 - 1986	Vice President, Biological Laboratories Division, Hazleton Biotechnologies, Kensington, Maryland.
1984 - 1985	Vice President, Biological Safety Evaluation Directorate, Litton Bionetics, Inc., Kensington, Maryland.
1981 - 1984	Vice President, Molecular Sciences Directorate, Litton Bionetics, Inc., Kensington, Maryland.
1974 - 1981	Director, Department of Molecular Toxicology, Litton Bionetics, Inc., Kensington, Maryland.
1971 - 1974	Assistant Professor of Microbiology, College of Medicine, Howard University, Washington, D.C.
1970 - 1971	National Academy of Sciences, National Research Council Postdoctoral Research Associate, Genetic Toxicology Branch. U.S. Food and Drug Administration, Washington, D.C.
1968 - 1970	Graduate Research and Teaching Assistant, Department of Biology, Illinois State University, Normal, Illinois.
1967 - 1968	Assistant Professor of Biology, Bridgewater College, Bridgewater, Virginia.

1963 - 1967	Graduate Research and Teaching Assistant, Department of Biology, Illinois State University, Normal, Illinois.

ACADEMIC APPOINTMENTS

1981 - Present	Adjunct Associate Professor in the Department of Biological Sciences, George Washington University, Washington, D.C.
1985 - Present	Adjunct Associate Professor in the Department of Genetics and Human Genetics, Howard University, College of Medicine, Washington, D.C.

EXPERIENCE

Scientific Director, Corning Hazleton Inc., Vienna, VA. Manager of mammalian toxicology and pathology sciences.

Principal Investigator on mutagenicity testing contracts from agencies of the Federal government (e.g. EPA, FDA, NIEHS, NIOSH, DOD) and private sponsors.

Research experience in mutagenicity of chemical carcinogens and other environmental agents, carcinogen mechanisms. Research included *in vitro* and *in vivo* investigations.

Scientific Director of mutagenicity testing and molecular toxicology for Hazleton Laboratories worldwide.

Member of the editorial board of four scientific journals in genetics and toxicology.

Editor of *In Vitro* Toxicology, an international journal published by Mary Ann Liebert, Inc. (1988-1993).

Member of the two U.S. National Academy of Sciences Committees with Mutagenesis and Toxicology (Diesel Impact Committee and Toxicology Data Elements Committee). Chairman of a NRC subcommittee on DNA adducts.

Member of the International Commission for Protection Against Environmental Mutagens and Carcinogens (1986 - present). Chairman 1989 - 1995.

Past President of the U.S. Environmental Mutagen Society (1978).

Panel Member of the U.S.-Japan Environmental Mutagen Cooperative Program (1977-1979).

Councillor to the EMS Society.

Member of the Steering Committee for the EPA on the Gene-Tox Program for Genetic Testing Evaluation.

Member of NIH Study Section on Toxicology, 1992-1996.

Consultant to government agencies and private industrial firms regarding mutagenesis testing.

Member of the Center for Alternatives to Animal Testing (CAAT), Technology Transfer Committee.

Board Member, Academy of Toxicological Sciences (1990-1993).

Board Member, Industrial *In Vitro* Toxicology Group (1989-present).

Secretary/Treasurer, Academy of Toxicological Sciences (1995-1996).

Associate Editor for Toxicological Sciences (1998 - present).

Rick D. Cardwell, Ph.D.

Ph.D., Fisheries (Aquatic Toxicology), University of Washington (1973)
Master of Science, Fisheries (Fish Physiology), Univ. of Washington (1968)
Bachelor of Science, Fisheries Science, Oregon State Univ. (1967)

Rick Cardwell is an aquatic biologist and ecotoxicologist by training with more than 25 years experience studying the effects of chemicals and habitat changes on aquatic life and wildlife. He has used a variety of tools for this purpose, including risk and impact assessment, field studies, monitoring, aquatic toxicity bioassays, computer modeling, and statistics. He has managed hundreds of projects in his career, several totalling more than $1.75 million in studies annually.

Project Experience (International)

Human Health and Ecological Risk Assessment for Copper Mine - PT Freeport Indonesia (1997)
Rick Cardwell is participating in a multi-disciplinary assessment of the risks posed by chemicals and sediments to people and their food supply downstream of a copper mine in Irian Jaya, Indonesia.

Strickland River Risk Assessment - Porgera Joint Venture, Porgera, Papua New Guinea
Rick Cardwell is managing a screening-level risk assessment of the risks posed to people and their fish and wildlife food supply downstream of a gold mine in the central highlands.

Third Party Peer Review of Risks Posed to Humans and Their Environment Downstream of Gold Mine -Papua New Guinea (1996)
Participated as member of third party, international team who reviewed the adequacy of existing environmental programs relative to potential risks posed by gold mining.

Risks Posed by Copper Mine Tailing in Marine Seafood- Lihir Island Copper Mine, Solomon Islands (1995)
Are the fish safe to eat? This is the question addressed in a human health risk assessment of fish and shellfish caught in the vicinity of a submarine discharge of effluent from a copper mine on Lihir Island. In addition to assessing risks, we estimated bioavailability and bioaccumulation of copper in marine food chains.

Risks Posed by Marine Outfall - Haifa Chemicals, Ltd., Israel (1994-1995)
Dr. Cardwell headed a project that evaluated the risks posed to marine life by heavy metals, pH and fluoride in effluent from a fertilizer plant in Israel. These included meetings with governmental officials, a screening-level risk assessment, evaluations of the chemical fate of effluent constituents, and evaluations of the effects posed by similar discharges elsewhere in the world.

Risk Assessments of Municipal Effluents, Sewer Overflows and Stormwaters - Sydney Water Corp., Sydney, Australia (1993-1997)
Conducted a series of human health and ecological risk assessments of various primary, secondary and tertiary-treated sewage, sewer overflows, and stormwaters in the Sydney, Australia metro area for the governing agency, Sydney Water Corporation. We assessed risks to people swimming and consuming fish and shellfish from local waters. Acute and chronic effects on aquatic life were also assessed. More than 100 metals, pesticides, and bacteria/viruses were evaluated, as were non-chemical stressors (suspended solids/turbidity, sedimentation, scour, and low dissolved oxygen).

Risk Assessment of Sewage Re-Use - Sydney Water Board, Sydney, Australia (1992)
Developed human health risk assessment of tertiary-treated sewage used as make-up water for a metal smelter in Sydney, Australia for the Water Board. Assessed risks to workers associated with exposure to metals, chlorinated organics (e.g., chloroform), and pathogenic bacteria/viruses in sewage.

Project Experience - (U.S.)

Literature Review Concerning the Fate and Transport and Bioavailability of Metals in the Aquatic Environment, International Council on Metals in the Environment (ICME)
Prepared a technical monograph synthesizing the literature concerning the fate, transport, bioavailability and bioaccumulation of metals in aquatic environments as it related to aquatic life.

Synthesis of Knowledge Concerning the Fate and Effects of Copper in the Environment - Kennecott Utah Copper (1994)
Copper is one of several metals whose environmental risks can be over-estimated if its biological availability is not defined. Accordingly, we reviewed and synthesized the scientific literature concerning the fate of copper in aquatic environments. Particular attention focused on its bioavailability, in water and sediments, and how bioavailability related to its bioaccumulation into -- and toxicity to -- aquatic organisms.

Development of Methodologies for Assessing Risks from Historic Copper Mining - ARCO Copper Mine, Butte, Montana (1992)
Retained by ARCO to assist multi-consultant team in developing ecological risk assessment methodologies for characterizing risks and identifying appropriate remediation for more than 20 riverine sites contaminated with wastes from historic mining operations near Butte, Montana.

Deciding When a River's Biological Health Has Been Restored Sufficiently: Historic Zinc Mine - Eagle River, Colorado (1993-present)
Conducting field studies of fish and aquatic invertebrates to define when the river had returned to biological health after years of impact due to acid rock runoff from historic zinc mining upstream. Analyzed factors affecting fish and invertebrates, including zinc, cadmium, and iron concentrations, stream flow, substrate composition.

Environmental Impact of Proposed Molybdenum Mine on Freshwater and Marine Life - U.S. Borax, Alaska (1983-1985)
Headed team of biologists who evaluated potential effects, as part of an environmental impact statement, of a proposed molybdenum mine situated with a U.S. national monument south of Ketchikan, Alaska. Evaluated impacts of sedimentation and water quality (especially heavy metals) on salmon in local streams and of marine organisms in the neighboring fjords.

Impacts of Antimony in Sediments and Surface Water Downstream of Antimony Smelter - Anzon, Inc., Laredo, Texas (1993)
Assessed impacts on aquatic life and human health uses from antimony in stream sediments and surface water. Conducted aquatic toxicity tests of antimony to establish site-specific water quality criteria, conducted sediment toxicity tests, analyzed dissolved vs. total recoverable antimony, and presented expert testimony. Prepared expert testimony in preparation for litigation.

Risks Posed by Metals, Arsenic and Selenium - Kennecott Utah Copper, Utah (1994-Present)
Risks posed by groundwater entering surface waters and contaminating the aquatic life consumed by various bird species was the question addressed in work for Kennecott Utah Copper, Inc. Additional assignments have involved development of water quality criteria for the Great Salt Lake, and revision of the national water quality criterion for selenium. A variety of metals (e.g., cadmium, copper) and metalloids (arsenic and selenium) were evaluated with respect to their bioaccumulation, bioavailability, and effects on shorebirds. Also conducted chronic tests with arsenic and selenium to evaluate toxicity and bioaccumulation in brine shrimp eaten by a variety of birds.

Site-Specific Water Quality Criteria, Lead Mines and Smelter - Missouri (1990)
Developed site-specific, surface water quality criterion for thallium, and attempted to develop site-specific criterion for cadmium and lead downstream of two lead mines and one smelter. The site-specific criterion for thallium, based on human health risks from consuming local fish, was three-times higher than the state standard.

Toxicity Identification Evaluations at Copper, Lead, Silver and Zinc Mines - ASARCO (1993-1994)
Conducted toxicity identification evaluations (TIE) of wastewaters from copper-silver and lead-zinc in Montana and Colorado, respectively. These TIEs included routine and customized toxicity testing and consulting in the causes of toxicity and methods for wastewater treatment.

Wildlife and Human Health Risk Assessments: Pit Lake Created from Gold Mining - Newmont Mining Co. (Formerly Santa Fe Pacific Gold Corp. , Nevada (1994-1995))
Parametrix assessed risks posed to wildlife and human health from exposure to the water, aquatic life, and plants colonizing a pit lake created via groundwater infiltration. Risks from several post-closure operations were assessed. Examined risks posed by metals, metalloids, and ions (Na, Mg, K, etc.).

Mercury in Estuarine Sediments - Lavaca Bay, Texas (1995-1998)
Conducted a risk-based remedial investigation/feasibility study of estuarine sediments contaminated with mercury in from Texas. Examined risks from mercury, polynuclear aromatic hydrocarbons, and polychlorinated biphenyls to freshwater and estuarine fish, invertebrates and wildlife (birds).

Metal Released from Lead Battery Recycling Site - Exide, Inc., Reading, Pennsylvania (1994-present)
Metals were released to the soil then to groundwater from recycling batteries at a site next to the Schuylkill River in Pennsylvania. Parametrix designed and conducted the chemical and ecological monitoring of potential adverse effects from lead and other heavy metals on river life.

Biological Impact Assessment of Arsenic - Elf Atochem North America, Bryan, Texas (1992-1996)
Designed and initiated a multi-year evaluation of the ecological impacts of historic arsenic releases in the metro Bryan-College Station, Texas area, including impacts to fish populations and invertebrates. Studies assessed arsenic concentrations, arsenic species, fish and invertebrate populations, and acute and chronic toxicity of surface waters and sediments.

Human Health Risks from Lead in Sediments and Surface Water - Hecla, Asarco, Coeur d'Alene Mining Corp. and Sunshine Mining Co. (1993)
Dr. Cardwell headed a team that assessed potential risks to human health from drinking water, eating dirt (children), and consuming sport-caught fish from the lower Coeur d'Alene River and Lake Coeur

d'Alene, Idaho, where lead, from historic lead mine tailings, frequently reached 10,000 ppm in the sediment (jig tails).

Site-Specific Water Quality Criteria: Metals – U.S. general
On behalf of the Electric Power Research Institute and Utilities Water Act Group, prepared comments and alternatives to methodologies proposed by both the U.S. EPA and Maryland for the derivation of site-specific water quality criteria for heavy metals and other toxic substances.

CURRICULUM VITAE

Charles H. Emerson, M.D., F.A.C.P. Telephone 508 856 3166

CURRENT POSITIONS - University of Massachusetts School of Medicine, Worcester, MA

Professor of Medicine

Professor of the Physiology Program

Associate Director: Endocrine Training Program

Associate Director: University of Massachusetts Medical Center Clinical Research Center

[redacted]

OFFICE ADDRESS

University of Massachusetts Medical Center
55 Lake Avenue North
Worcester, Massachusetts 0l655

[redacted]

EDUCATION

High School Diploma 5/5/59
Kodaikanal School, Kodaikanal, India 1955-1959

B.S. Received 6/2/63
Randolph Macon College, Ashland, VA 1959-63

M.D. Received 6/4/67
University of Virginia Medical School, Charlottesville, VA 1963-67

POSTDOCTORAL TRAINING

Intern in Medicine
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
6/23/67 to 6/24/68

Resident in Medicine
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
7/1/68 to 6/30/70

Fellow in Endocrinology and Metabolism
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania
7/1/70 to 6/30/71 and
7/1/73 to 6/30/74

LICENSURE

Inactive Licenses
6/23/67 - State of Virginia, License Number 18582
6/17/74 - State of Illinois, Certificate Number 36-49327

Active Licenses
3/31/80 - State of Massachusetts (Certificate 45591)
5/16/97 - State of Connecticut (Liscense No. 036013)

Specialty certification
6/21/72 - Internal Medicine, Certificate No 35149
10/18/77 - Endocrinology and Metabolism, Certificate No 35149

ACADEMIC APPOINTMENTS

7/1/68 to 6/30/70 - Assistant Instructor in Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

7/1/70 to 6/30/71 -	Postdoctoral Fellow University of Pennsylvania School of Medicine
1/1/72 to 6/30/73 -	Clinical Instructor in Medicine Medical College of Georgia, Augusta, Georgia
7/1/73 to 6/30/74 -	Postdoctoral Fellow University of Pennsylvania School of Medicine
7/1/74 to 3/31/80 -	Assistant Professor of Medicine, Abraham Lincoln School of Medicine (ALSM) of the University of Illinois, Chicago, Illinois
4/1/80 to 4/5/86 -	Associate Professor of Medicine, University of Massachusetts School of Medicine, Worcester, MA
2/6/86 to present -	Professor of Medicine, University of Massachusetts School of Medicine, Worcester, MA
12/14/87 to present -	Professor of Medicine, University of Massachusetts School of Medicine, and Professor of the Physiology Program, University of Massachusetts Graduate School of Biomedical Sciences, Worcester, MA

PROFESSIONAL/ADMINISTRATIVE APPOINTMENTS

U.S. Army Medical Center, Fort Gordon, GA

7/6/71 to 6/30/73 - Chief, Endocrine Service and Staff Physician in Medicine

University of Illinois Hospital, Chicago, IL

7/1/74 to 9/1/78 - Staff Physician in Medicine VA West Side Hospital, Chicago, IL

7/1/74 to 9/1/78 - Staff Physician in Medicine and Nuclear Medicine

University of Massachusetts Medical Center, Worcester, MA

10/31/80 to 12/30/82 - Staff Physician in Medicine and Nuclear Medicine

1/1/83 to 8/1/83 - Staff Physician in Medicine and Nuclear Medicine Acting Chief, Division of Endocrinology and Metabolism, Department of Medicine

The Children's Hospital Medical Center, Boston, MA

10/18/89 to 10/18/93 - Visiting Staff (Research)

University of Massachusetts Medical Center, Worcester, MA

8/1/83 to present - Staff Physician in Medicine and Nuclear Medicine, Coordinator: Training Programs in Endocrinology, Metabolism and Diabetes

MEMBERSHIP IN HONORARY SOCIETIES

1963 - Chi Beta Phi Honorary Scientific Fraternity

1966 - Alpha Omega Alpha Honorary Medical Fraternity

1963 to 1967 - Florence Smith Scholar

CURRICULUM VITAE

Name: Joseph K. Haseman

Date and Place of Birth: July 20, 1943; Sheffield, Alabama

Citizenship: United States [redacted]

Education:

June, 1961 Graduated from High School (Valedictorian)
June, 1965 B.S. (Mathematics) cum laude, Davidson College
June, 1970 Ph.D. (Biostatistics), University of North Carolina (Chapel Hill)
Dissertation: The Genetic Analysis of Quantitative Traits Using Twin and Sib Data

Brief Chronology of Employment:

1966 - 1966 Summer employment as a statistician and computer programmer, Research Triangle Institute, Research Triangle Park, N.C.

1968 - 1970 Laboratory Instructor, Biostatistics Department, University of North Carolina, Chapel Hill, N.C.

1970 - date Research Mathematical Statistician, Biostatistics Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, N.C. 27709. Phone: (919) 541-4996. Fax: (919) 541-4311.

Professional Societies:

Biometric Society (ENAR), American Statistical Association (ASA), Society of Toxicology, Genotoxicity and Environmental Mutagen Society, Phi Beta Kappa

Honors and Professional Recognition

NIH Director's Award, 1983
Associate Editor, Shorter Communications, Biometrics, 1979-1984.
ASA Biopharmaceutical Section Executive Committee, 1987-1989.

Board of Editors, Environmental Health Perspectives, 1980-1997.
Editorial Board, Fundamental and Applied Toxicology, 1986-1992.
Elected as Fellow, American Statistical Association, 1989.
Distinguished Achievement Medal, American Statistical Association's
Section on Statistics and the Environment, 1994.

Honored by Toxicologic Pathology for having one of the 10 most frequently cited articles in that journal over its 25 year history, 1997.

February 1998

CURRICULUM VITAE

Susan P. Porterfield, Ph.D.

Professor of Physiology and Endocrinology
Associate Dean for Curriculum
Office Address: CB-1104; CL-3150
Office Telephone: (706) 721-3217

[redacted]

Education

High School: Worthington High School, Worthington, Ohio, graduated 1961

College: Miami University, Oxford, Ohio, 1961-1963
Ohio State University, Columbus, Ohio, 1963-1965; B.S. degree
Summa Cum Laude; Physiology, Chemistry

Graduate Education: Ohio State University, Columbus, Ohio, 1965-1967;
M.S. Degree 6/67; Physiology
Ohio State University, Columbus, Ohio, 1971-1973;
Ph.D. Degree 12/73; Physiology

Academic Appointments

Research Associate, Ohio State University, 1967
Laboratory Instructor, Ohio Northern University, Ada, Ohio, 1969
Instructor, Ohio Northern University, Ada, Ohio, 1970-1973
Assistant Professor, Georgia Institute of Technology, Atlanta, Georgia, 1973-1976
Assistant Professor, Medical College of Georgia, Augusta, Georgia, 1976-1979
Associate Professor, Medical College of Georgia, Augusta, Georgia, 1979-1996
Professor, Physiology & Endocrinology, School of Graduate Studies, Medical College of Georgia, 1996-present
Associate Dean for Curriculum, Medical College of Georgia November 1994-present

Administrative Appointments

Associate Dean for Curriculum November 1994-present

Editorial Boards

Endocrine Reviews - 1992-1996

Outside Reviewer

Avline Reviewer
Journal of Nutrition
Endocrinology
Endocrine Reviews
New England Journal of Medicine
Environmental Health Perspectives
Journal of Clinical Endocrinology

Ad Hoc Grant Reviewer

National Science Foundation 1990
NIH - endocrine study section 1995, 1996
EPA 1996
Human Frontier Science Program (HFSP) 1996

Abstract Reviewer

Endocrine Society, 1987
American Physiological Society, 1984
Endocrine Society, 1997

Awards/Honors

Summa Cum Laude, Ohio State University
Member of *Phi Beta Kappa*
Recipient of a Muhlhaupt Fellowship (Ohio State), 1965-1966 and 1966-1967
President's Scholarship - Miami University
President's Award - Ohio State University
Participant - Honors Program, Miami University
Who's Who in the Southeast
Outstanding Faculty Award for Basic Science Teaching, 1993
Selected as a member of the National Board of Medical Examiners, Physiology Test-writing Committee Step I, 1994-present
Selected to serve as a member of the National Board of Medical Examiners, Physiology subject exam writing committee, 1994-present
Session Chairperson, National Meetings

1. American Physiological Society, Fall Meeting, Endocrinology & Metabolism, 1984
2. Endocrine Society, Thyroid Hormone Action, 1987
3. Developmental neurotoxicity of endocrine disrupters, 13th International Neurotoxicology Conference, 1995

4. Integration of clinical skills in basic science courses, Generalist Physician Initiative, 2nd Annual Program, Key Biscayne, FL
5. American Thyroid Association, Colorado Springs, CO, 1997

Invited faculty for the International Symposium on Advances in Perinatal Thyroidology at Longboat Key, FL, 1990. My participation was supported by Boots Pharmaceutical.

Invited faculty for the Learning Disorders Association/EPA, NIEHS and National Foundation for Brain Research sponsored conference "The Decade of the Brain", 1992. My participation was supported by the EPA.

Invited to serve as professional consultant to the EPA in the Learning Disorders Association Round-Table 1992, 1995.

Invited by Dr. Sumner Jaffee, Director for Research in Maternal and Child Health, NICHD, to participate and present in a workshop to develop research ideas on studying the impact of thyroid disorders on early fetal brain development. Conference was June 27, 28, 1994. My participation was supported by the NICHD.

Invited faculty for the Learning Disorders Association conference on "Thyroid Function and Learning Disabilities" to be held March 2, 1995. My participation will be sponsored by the Learning Disorders Association.

Invited speaker/participant, Conference on steroid hormones and the brain; Breckenridge, CO, March 31-April 4, 1995.

Invited consultant for the EPA; workshop on environmental toxins, thyroid hormones and brain development held in Raleigh on April 10-14, 1995

Invited speaker/participant for a conference on "Effects of Xenobiotics on Development of the Nervous System" held November 2-9, 1996 in Italy. My participation was be sponsored by NATO, and the Italian government.

Invited speaker/participant International Neurotoxicology Conference--Developmental neurotoxicity of endocrine disrupters, Hot Springs Ark, Oct. 1995, sponsored and supported by the EPA.

Selected as a participant at the Association of American Medical Colleges Professional Development Seminar for Senior Women in Medicine, Washington, D.C., March 25-27, 1995.

Selected as a participant at the Association of American Medical Colleges Executive Development Seminar for Associate Deans and Department Chairs, Ft. Lauderdale, FL, November 16-20, 1996.

Invited participant International Endocrine Disrupters Workshop sponsored by the Smithsonian Institution, the White House Office of Science and Technology Policy, United Nations Environment Program and United States Environmental Protection Agency. The workshop was January 23-24, 1997, in Washington, DC.

Invited speaker International Workshop on "Effects of endocrine disrupters in the environment on neuronal development and behavior--current knowledge, assessment, gaps." The workshop is sponsored by the German Environmental Protection Agency. The workshop was February 17-18, 1997 in Berlin, Germany.

Invited speaker – "Development, Function, and Teratology of the Thyroid", Development and Function of Endocrine and Immune Systems in Teratology, sponsored by the Teratology Society, San Diego, CA, June 20, 1998

Invited speaker -- "Xenobiotics and Thyroid Function"; The Role of Environmental Neurotoxicants in Developmental Disabilities, The 20th Rochester Conference on Environmental Toxicity, sponsored by the NIEHS, Rochester, NY September 23-24, 1998.

Invited speaker - "Perinatal Thyroid Function", NIH sponsored international conference on perinatal endocrinology, Nancy, France, September, 1998

Invited speaker - Learning Disabilities Association, Annual Meeting, Atlanta, GA, February, 1999.

Scientific and Professional Societies

Member Georgia Academy of Science
Member of American Association for the Advancement of Science
Member of the Endocrine Society
Member of the Society for Experimental Biology and Medicine
Member of the American Physiological Society
Member of the American Thyroid Association
Member of the Southeastern Society of Experimental Biology and Medicine

ROCHELLE W. TYL, Research Director, Center for Life Sciences and Toxicology

Professional Experience

Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, NC.

7/15/93 - present. Research Director, Center for Life Sciences and Toxicology. Establish and implement Center goals and objectives consistent with RTI's overall financial and research strategic planning; recommend, interpret, and implement RTI policies and procedures; establish guidelines and procedures for and manage allocation and utilization of Center facilities, equipment, and funding; initiate, plan, and implement operational plans and strategies for Center direction(s) and development, and staff professional development; play a major role in establishing and implementing marketing goals and activities for the Center; maintain high level of involvement in technical efforts of the Center, including establishment and implementation of objectives, goals, and procedures of projects, coordination and cooperation among senior staff in complex projects; contribute to and direct preparation and presentation of research reports to clients and the professional community, in addition to duties as described below. Direct supervision of six PhDs, one DVM, and four senior technical staff; Center is comprised of 43 staff.

5/1/91 - 7/14/93. Assistant Research Director, Mammalian Toxicology Program; Program Manager, Reproductive and Developmental Toxicology. Duties as described below.

5/1/90 - 4/30/91. Senior Program Director, Mammalian Toxicology Program; Program Manager, Reproductive and Developmental Toxicology. Duties as described below. In addition, supervise senior staff in general toxicology and adult and developmental neurotoxicology testing, work with higher management in policy and program development and implementation, building and renovation program for CLS.

6/15/89 - 4/30/90. Program Manager, Reproductive and Developmental Toxicology. Supervision of the staff of Developmental Toxicology, Developmental Neurotoxicology, Reproductive Toxicology, and Reproductive Endocrinology. Market and perform reproductive and developmental toxicity evaluations for governmental, industrial, and commercial clients according to appropriate governmental guidelines, FDA, EPA (FIFRA or TSCA), OECD, EPA Test Rules or FIFRA Data "Call-Ins," under Good Laboratory Practice regulations; design and implement research protocols in reproductive and developmental toxicology for various clients; plan, coordinate, and direct activities in program; prepare bids, protocols, final reports and manuscripts for refereed journals; evaluate personnel performance; direct supervision of four Ph.D. staff, two senior technical supervisory staff, and an administrative assistant. Program includes seven doctoral level staff, six supervisory staff and approximately 15 technical staff. Member of RTI Institutional Animal Care and Use Committee (IACUC), 1990 - present.

9/1/87 - 6/89. Assistant Director, Reproductive and Developmental Toxicology, Bushy Run Research Center (BRRC), Export, PA. Work with the Director and Associate Directors in planning, coordinating, and directing work activities at BRRC. Prepare equipment and personnel budgets and evaluate personnel performance. Supervise a postdoctoral scientist. Additional responsibilities as below.

7/1/83 - 8/31/87. Manager, Reproductive and Developmental Toxicology Section, Bushy Run Research Center (BRRC), Export, PA. Testing for developmental and reproductive toxicity of industrial chemicals by all routes of exposure in rats, rabbits, and mice for Union Carbide and other industrial clients. Supervise ten staff members, responsible for protocol development, contact with clients, writing SOPs, writing study reports and manuscripts for refereed journals, staff training, sponsor liaison for UCC teratology studies done elsewhere, work under GLPs, FDA, EPA (TSCA), EPA (FIFRA), OECD guidelines, presentation of data to various professional groups.

5/83 - 6/30/83. Manager, Teratology Section and Senior Research Teratologist I, Research Triangle Institute, Research Triangle Park, NC. Research and testing in teratogenicity and reproductive toxicity of food additives, drugs, industrial chemicals, environmental pollutants, etc., in rats, mice, and rabbits for governmental and industrial clients. Supervise eight people, responsible for all facets of studies, client contact, protocol development, SOPs, writing reports and manuscripts for refereed journals, Institute and community service by lectures to professional and academic groups, work under GLPs, EPA, FDA guidelines.

7/81 - 5/83.. Supervisor, Teratology Section and Senior Research Teratologist I, Research Triangle Institute, Research Triangle Park, NC. Research and testing in teratogenicity and reproductive toxicity of food additives, drugs, industrial chemicals, environmental pollutants, etc., in rats, mice, and rabbits for government and industrial clients. Supervise eight people, responsible for all facets of studies, client contact, protocol development, SOPs, writing reports and manuscripts for refereed journals, Institute and community service by lectures to professional and academic groups, work under GLPs, EPA, FDA guidelines.

7/78 - 7/81. Head, Teratology Section, Chemical Industry Institute of Toxicology (CIIT), Research Triangle Park, NC. Research and testing in teratogenicity and reproductive toxicity of priority commodity chemicals, toxicokinetics, and metabolism of commodity chemicals in pregnant animals, development of test battery for postnatal sequelae of *in utero* exposures.

9/68 - 6/78. Assistant Professor (1968-1973), Associate Professor (1973-1978), University of Connecticut, Southeastern Campus, Groton, CT. Teaching undergraduate courses in Biological Sciences, Genetics, Embryology, advising independent research students (undergraduate) and Master's thesis research students, direct own research, premedical student counseling, university committee memberships (tenured, 1974).

Education

A.B. cum laude, Biological Science, University of Connecticut, Storrs, CT, 1963
Ph.D., Developmental Genetics, University of Connecticut, Storrs, CT, 1968

Professional Societies

Teratology Society
Neurobehavioral Teratology Society
Society of Toxicology (SOT) Active Member
SOT, Reproductive and Developmental Toxicology Subsection Member
Middle Atlantic Reproduction and Teratology Association (MARTA)
North Carolina Chapter of SOT
Genotoxicity and Environmental Mutagenesis Society (GEMS, NC)
Allegheny-Erie Regional Chapter of SOT (1983-1989)

Honors

Phi Beta Kappa
Phi Kappa Phi
Sigma Xi
Gamma Sigma Delta

Certification

Diplomate, American Board of Toxicology (ABT), 1983; recertified in 1988, 1993, and 1998

CURRICULUM VITAE

Name	Kimber Littlepage White, Jr.

[redacted]

Date of Birth	May 2, 1950
Place of Birth	Boston, Massachusetts
Citizenship	United States

[redacted]

Office Address	527 North 12^{th} Street Strauss Immunotoxicology Research Laboratory Room 2011 Medical College of Virginia/Virginia Commonwealth University Richmond, Virginia 23298 (804) 828-6789 Phone (804) 828-5604 FAX
Military Service	United States Navy, 1968-1977
Rank:	Lieutenant
Qualifications:	Surface Warfare Officer Command Duty Officer Officer of the Deck Underway (Fleet) Engineering Officer of the Watch Combat Information Center Watch Officer
Awards:	Combat Action Ribbon Meritorious Unit Citation Vietnam Campaign Medal Vietnam Service Medal Letter of Commendation for Lifesaving
Vietnam Service:	1972-1973

CURRICULUM VITAE
Kimber Littlepage White, Jr.

Educational Background

Ferguson High School, Newport News, Virginia	1968
B.S., United States Naval Academy Annapolis, Maryland Chemistry	1972
Ph.D., School of Basic Sciences Department of Pharmacology and Toxicology Medical College of Virginia Virginia Commonwealth University Richmond, Virginia	1981

Thesis Title: "Immunotoxicology of Chrysotile Asbestos"

Honors

NIH Predoctoral Fellow 1978 - 1981

Positions Held

5/1981-12/1982	Research Associate and Adjunct Assistant Professor Department of Pharmacology and Toxicology Medical College of Virginia/VCU
1/1983 - 8/1984	Assistant Professor Department of Microbiology and Immunology Medical College of Virginia/VCU
	Assistant Professor (Affiliate) Department of Pharmacology and Toxicology Medical College of Virginia/VCU
9/1984 - 7/1991	Assistant Professor Department of Biostatistics Medical College of Virginia/VCU
	Assistant Professor (Affiliate) Department of Pharmacology and Toxicology Medical College of Virginia/VCU
7/1986 - 7/1991	Assistant Professor (Affiliate) Biomedical Engineering Program Medical College of Virginia/VCU
7/1991 - 7/1995	Associate Professor Biomedical Engineering Program Medical College of Virginia/VCU

CURRICULUM VITAE
Kimber Littlepage White, Jr.

Positions Held (Continued)

Associate Professor (Affiliate)
Department of Pharmacology and Toxicology
Medical College of Virginia/VCU

7/1995 - present — Associate Professor
Department of Pharmacology and Toxicology
School of Medicine
Medical College of Virginia/VCU

Associate Professor (Affiliate)
Biomedical Engineering Program
School of Engineering
Medical College of Virginia/VCU

Membership - Scientific, Honorary, and Professional Societies

Society of Toxicology - Immunotoxicology Subsection
Chairman, Methods Committee 1988-89, 1989-90
Councilor 1991-1993
- Risk Assessment Subsection
- National Capital Area Chapter

Immunotoxicology Discussion Group
Steering Committee Member 1991-present
The American Association of Immunologists
American Association for the Advancement of Science
Virginia Academy of Science

Invited Speaker or Invited Participant

1981 — International Research and Development Corporation
Symposium on Immunotoxicology

1984 — Environmental Protection Agency
Office of Pesticides and Toxic Substances

Immunotoxicology Discussion Group
Critical Review of Current Methodology

Northeast Chapter of Medicinal Chemists

1985 — Environmental Protection Agency
Office of Pesticides and Toxic Substances

Northrop Services, Inc., Research Triangle Park

1986 — Sterling-Winthrop Research Institute

CURRICULUM VITAE

R. Thomas Zoeller
Department of Biology
University of Massachusetts, Amherst, MA 01003
Tel. (413) 545-2088 /// *Fax.* (413) 545-3243
Email: tzoeller@bio.umass.edu

Academic Appointments:

Associate Professor with tenure, Biology Department, University of Massachusetts at Amherst, '96-Present
Associate Professor, Biology Department, University of Massachusetts at Amherst, '94-'96
Assistant Professor, Dept Anat. & Neurobiol., Univ. Missouri-Columbia Sch. Med. '88-'94
Research Associate, Laboratory of Neurochemistry, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD , '87-'88
Postdoctoral Researcher, Laboratory of Cell Biology, National Institute of Mental Health, NIH, Bethesda, MD '84-'87.
Research Assistant, Department of Zoology, Oregon State Univ. Corvallis, '80-'84

Education:

B.S., Indiana University, Bloomington. 1977 (Biology)
M.A., Oregon State University, Corvallis. 1979 (Endocrinology)
Ph.D., Oregon State University, Corvallis. 1983 (Neuroendocrinology)
Postdoctoral fellow, LCB, NIMH, NIH, Bethesda, MD
NRC Research Associate, LNC, NINDS, NIH, Bethesda, MD

Affiliations:

Member:
- University of Massachusetts Center for Neuroendocrine Studies
- UMass Neuroscience and Behavior Program
- UMass Molecular and Cellular Biology Program
- UMass Organismal and Evolutionary Biology Program
- American Association for the Advancement of Science
- Endocrine Society
- Society for Neuroscience
- Research Society on Alcoholism
- International Brain Research Organization
- International Society for Biomedical Research on Alcoholism

Honors:

Best Student Paper Award, American Society of Zoologists '82
Oregon State University Bayley Graduate Fellow, '82-'83.
Individual National Research Service Award (1984-1987), National Institute of Mental Health, NIH.
Individual Resident Research Associateship (1987-1988), National Institute of Neurological Disorders and Stroke. Awarded by the National Research Council of the National Academy of Sciences
Invited Participant, 2nd ORPRC Symposium on Primate Reproductive Biology: Neuroendocrine Aspects of Reproduction. Oregon Regional Primate Research Center, Beaverton, Oregon (1982).
Invited Speaker, "Reproductive Neuroendocrinology, New Approaches to Old Questions", San Antonio, TX, 27 December, 1990.
Invited Discussant, Ciba Foundation Symposium #168, "Functional Anatomy of the Neuroendocrine Hypothalamus", 8-10 October, 1991, Budapest, Hungary.
Invited Speaker and Chair, Session on "Thyroid Hormones and Brain Function" 1995 Steroid Hormone Workshop, Breckenridge, CO

Military Service

Drafted June 14, 1972, Honorable Discharge June 13, 1974.

Professional Reviews:

Ad hoc Reviewer for the following journals:
American Journal of Physiology, Brain Research, Molecular Brain Research, Developmental Brain Research, Development, Endocrinology, Molecular Endocrinology, General and Comparative Endocrinology, Histochemistry, Journal of Neuroendocrinology, Journal of Neuroscience, Journal of Experimental Zoology, Neuroscience Journal, Regulatory Peptides

Ad hoc Reviewer for the following agencies
National Science Foundation, US Veteran's Administration, Human Frontier Science Program Organization, EPA.

Member, Screening and Testing Workgroup of the Endocrine Disruptor Screening and Testing Advisory Committee.

Meetings Organized

Thyroid Hormones and Brain Function, a session at the 1995 Workshop on Steroid Hormones and Brain Function, Breckenridge, CO.
23rd New England Endocrinology Conference, Amherst, MA (September, 1995).

Research Support History:

Bayley Graduate Fellow (Oregon State Univ., 1983-1984)
Individual National Research Service Award (MH09104; 1984-1987)
Individual Fellow of the National Research Council (1987-1992; accepted only 1 year of 5). "Molecular Analysis of Neuroendocrine Peptide Gene Expression"
State Research Council (UMC, 1988-1989; 4,645) "Molecular Analysis of Neuroendocrine Peptide Gene Expression"
Medical Research Council (UMC, 1989-1990; $25,000) "Neuroendocrine Peptide Gene Expression in Rat Brain"
American Heart Association (1990-1991; $30,000) "Cardiovascular Elements within the CNS: The Role of TRH Neurons" (T. Zoeller, PI).
Graduate Research Council, UMC (1990-1991; $3,340) "Central Regulation of Cardiovascular Function"
National Institutes of Health (RO1 AA 08887; 1991-1995; $281,300 direct) "Molecular Basis for Ethanol Induced Hypothyroidism" (T. Zoeller, PI) Note: I brought this grant with me from Missouri, but chose not to renew it as an alcohol grant (see statement of research direction). Rather, I have re-focused this work on an issue that this work revealed and have funded it through a separate mechanism (NSF IBN9514835).
National Institutes of Health (RO1 NS30178; 1992-1995; $411,925 direct) "IP3 metabolism and Ca++ homeostasis in cerebral ischemia (GY Sun, PI; T.Zoeller, 10% effort). Note: I have not maintained this collaboration after moving to UMass.
National Institutes of Health NRSA to NIAAA (1993-1996; $70,000 direct) "Ethanol and Hypothalamic-Pituitary-Thyroid Axis" (Sponsor for Dr. HC Scott). Note: Dr. Scott was not able to move with me to UMass for personal reasons. However, I continue to advise her on her work, and we have maintained a productive collaboration.
Graduate Research Council, Univ. Massachusetts (1994-1995; $5,000) "Identification of Thyroid Hormone-Regulation Genes in CNS Development". Note: This work has led directly to a collaboration with a group an UMass Worcester.
National Institutes of Health (RO1 AA10418; 1996-1999; $437,850, direct) "Molecular Mechanisms Underlying Fetal Alcohol Syndrome" (T.Zoeller, PI; Active).
National Science Foundation (IBN-9514835; 1996-1998; $80,000) "Functional Organization of Hypophysiotropic TRH Neurons" (T.Zoeller, PI; Awarded March 1, 1996, Active)

National Institutes of Health (ES083330; 1996-1999; $300,000 direct) "PCBs and Thyroid Hormone Action in Developing Cochlea" (T. Zoeller, PI; Active).

Appendix D

Conflict of Interest of Peer Reviewers

CONFLICT OF INTEREST

Each of the following Peer Reviewers signed a statement saying the following, "The undersigned hereby warrants that, to the best of their knowledge and belief, that no actual or potential organizational or personal conflicts of interests exist with respect to the perchlorates work assignment."

Dr. Melvin Andersen
Colorado State University

Dr. David Brusick
Covance Laboratories, Inc.

Dr. Rick Cardwell
Parametrix, Inc.

Dr. Charles Emerson
University of Massachusetts Medical Center

Dr. Joseph Haseman
National Institute of Environmental Health Sciences

Dr. Curtis Klaassen
University of Kansas Medical Center

Dr. Susan Porterfield
Medical College of Georgia

Dr. Rochelle Tyl
Research Triangle Institute

Dr. Kimber White
Medical College of Virginia

Dr. R. Thomas Zoeller
University of Massachusetts

Appendix E

Charge to External Peer Review Panel for Perchlorate Toxicity

1.0 Charge to External Peer Review Panel for Perchlorate Toxicity

1.1 Review of Individual Studies Initiated Since May 1997

For each study report assigned to you as a primary or secondary reviewer, please respond to the following questions:

1. Please comment on the strengths and weaknesses of the experimental design of the study. Are the questions being investigated in each study clearly identified? Are they important to enhancing the toxicological (ecotoxicological) characterization of perchlorate? Is the study design appropriate to answer the questions? Discuss all limitations in experimental design that would affect the ability to interpret the significance of study results. Also indicate areas in which insufficient information has been provided on the experimental design.

2. Please comment on any limitations in the conduct of the study which could decrease the relevance of study findings. For example, were the studies conducted in accordance with Good Laboratory Practices? Were there occurrences that necessitated a change in the protocol during the course of the study? If so, what impact did these changes have on the findings?

3. Please comment on the strengths and weaknesses of the statistical methodology(ies) used to evaluate study findings. What other statistical analyses, if any, should be performed?

4. Please comment on the strengths and weaknesses of the presentation of the investigations in the study report. Were sufficient data presented in the report and its appendices to confirm the findings presented in the report? Are the conclusions of the report supported by the data? Please explain.

5. Overall was the study as designed, performed and reported of sufficient quality for use for hazard characterization purposes? If so, indicate the extent to which it can be used for characterizing human health/ecotoxicological effects of ammonium perchlorate and the perchlorate ion. Do the findings provide information relevant to evaluating the sensitivities of specific subpopulations of exposed individuals and attendant effects (e.g., infants, hypothyroid individuals)?

6. For the studies that are not yet complete, are sufficient data available on experimental design, conduct and interim observations to derive meaningful conclusions? If so, what caveats, if any, should be placed on these conclusions? Or should all data from the study be evaluated following the conclusion of the study and development of the final study report?

1.2 Review of Toxicological Review Document

1.2.1 Effects of Concern to Human Health (all reviewers except Dr. Cardwell)

Please comment on the adequacy of the Toxicological Review document in presenting and evaluating the existing toxicology data base on ammonium perchlorate and the perchlorate ion relevant to effects on human health.

1. Have the key aspects of the protocols, conduct and results of each toxicology study been adequately described in the Toxicological Review document? Where limitations exist in study reports or published papers, have they been appropriately discussed in the Toxicological Review document? In what ways might the discussion of studies be improved?

2. Indicate the strengths and weaknesses of the analyses performed on the data in the Toxicological Review document first of specific toxicological studies and then of the overall toxicology data base on perchlorate. Has the document adequately evaluated the results of all relevant studies and the biological significance of the entire data base? Where inconsistencies appear to exist in the findings relevant to the hypothalamic-pituitary-thyroid axis within and between studies, does the document adequately address such inconsistencies? Enumerate specific improvements that should be made, if any.

3. Authors of the Toxicological Review document provided statistical analyses beyond those contained in the original study reports of recently completed studies. Where these statistical analyses were performed, were the appropriate methodologies used? Did they add to the overall understanding/relevance of the studies? Were the appropriate endpoints and/or time points used? Please explain.

4. Note any relevant references that have not been cited in the Toxicological Review document and their relevance to hazard characterization of ammonium perchlorate and the perchlorate ion.

1.2.2 Ecotoxicological Effects of Concern (Dr. Cardwell)

Please comment on the adequacy of the Toxicological Review document in presenting and evaluating the existing data base of ecotoxicological effects of ammonium perchlorate and the perchlorate ion.

1. Have the key aspects of the protocols, conduct and results of each study of ecotoxicological effects been adequately described in the Toxicological Review document? Where limitations exist in study reports or published papers, have they been appropriately discussed in the Toxicological Review document? In what ways might the discussion of studies be improved?

2. Indicate the strengths and weaknesses of the analyses performed on the data from individual studies of ecotoxicological effects and then of the overall data base on ecotoxicological effects of perchlorate. Has the document adequately evaluated the results of all relevant studies and the biological significance of the entire data base?

3. Note any relevant references on ecotoxicological effects of ammonium perchlorate or other perchlorate salts that have not been cited in the Toxicological Review document and their relevance to the characterization of ecotoxicological effects of these compounds.

1.2.3 Additional Issues Pertaining to the Toxicological Review Document (all peer reviewers)

1. Are there other sections of the document that could be improved? Please specify and note the revisions that would improve the document.

2. Is the document as currently written useful for the purpose of characterizing the human health/ecotoxicological effects of ammonium perchlorate and the perchlorate ion? If not, specify the nature and extent of changes that are needed.

1.3 Hazard Characterization

1.3.1 Development of Reference Dose (RfD) (all reviewers except Dr. Cardwell)

See the attached summary of the EPA guidelines for evaluating noncancer health effects of environmental chemicals through the development of reference doses (RfDs) and for evaluating carcinogenic effects through the development of cancer potency factors.

1. The Toxicological Review document developed no observed adverse effect levels (NOAELs) and/or lowest observed adverse effect levels (LOAELs) for most of the studies discussed in the document. Are the individual NOAELs/LOAELs appropriate given the totality of data from each study? Please explain.

2. It is general EPA policy to develop estimates of non-cancer toxicities of environmental chemicals by using the results on the most sensitive toxic endpoint from the group of toxicology studies that have been performed on the chemical. This serves as the basis for the reference dose(RfD), an estimate of a daily lifetime exposure without risk of deleterious noncancer effects during a lifetime. Given that all available data indicate that the thyroid organ is the most sensitive organ for perchlorate toxicity, it is important to assess the internal consistency of the overall data base. Comment on the use of a single study or the totality of data on thyroid toxicology as the basis for establishing an RfD.

3. The approach used in the Toxicological Review document for developing an RfD for perchlorate was to identify the principal study as the neurodevelopmental toxicity study in rats and the critical effect as the decrease in follicular lumen size and follicular cell

hyperplasia observed in pups on postnatal day 5 at the 0.1 mg/kg/day dose. Is this the appropriate selection? Is the designation of the 0.1 mg/kg/day dose as a "minimal" LOAEL an appropriate choice based upon the totality of the data? If not, specify a more appropriate approach to developing an RfD based upon the current toxicology data base.

4. The EPA position, as stated in the document entitled "Assessment of Thyroid Follicular Cell Tumors," is that in the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption. This is considered to be a public health protective position where thyroid-pituitary disruptions are the sole mode of action, because rodents appear to be more sensitive to this carcinogenic mode of actions than humans. The available data base on the perchlorate ion indicates that disruption of the thyroid-pituitary axis is the most sensitive endpoint. Further, long-term treatment with perchlorate induced benign thyroid tumors in rats. Moreover, perchlorate appears not to be genotoxic based upon available data. Given the above EPA position and the toxicity data base on perchlorate, is the choice of an uncertainty factor of 3 for extrapolating potential differences in iodide inhibition between rodents and humans appropriate in the derivation of the RfD? If not, what should the uncertainty factor be for interspecies extrapolation?

5. Additional uncertainty factors of 3 each were used to account for using a minimal LOAEL as opposed to a NOAEL and to partially address intrahuman variability in pharmacodynamics, for data base deficiencies, and for accounting for intrahuman variation (sensitive subpopulations) in iodide uptake inhibition. Identify the strengths and limitations of using 3 as the value for each of these uncertainty factors. Would other values (e.g., 10 or 1) have been more appropriate? If so, specify those values and the reasons for their selection.

6. The Toxicological Review document concludes that the "RfD" is actually a harmonized oral human health risk estimate that will be protective for both noncancer health effects and cancer endpoints of perchlorate ion since the RfD is based upon reversible effects observed at dose levels below those at which thyroid tumors or neurodevelopmental effects were induced in the rat studies. Do the existing data support this position? Please explain.

1.3.2 Ecotoxicological Assessment (Dr. Cardwell)

1. Comment on whether the goals and objectives of this ecological screening analysis have been adequately described and to what extent these have been met.

2. Does the analysis support the summary and conclusions presented? Are relevant and important aspects of uncertainty are addressed sufficiently? Which aspects are not, and how could the discussion be improved?

3. Comment on whether the assays selected for evaluation in the ecological screening analysis can be reasonably expected to identify potential ecological effects of concern.

1.4 Further Testing Needs for Perchlorate

1.4.1 Toxicological Testing (all members of the peer review panel except Dr. Cardwell)

1. Were the experimental designs of the toxicity studies undertaken since May 1997 adequate to identify the potential hormone disrupting effects on development and reproductive performance due to thyroid function perturbations at low exposure levels? If not, specify more appropriate protocols.

2. Identify additional toxicology studies that would lead to a more complete toxicological characterization of ammonium perchlorate and the perchlorate ion. Provide information on the protocols that should be utilized.

3. Comment on the potential value added to these analyses by the development of a physiologically-based pharmacokinetic model to address species differences in inhibition of iodide uptake, perchlorate kinetics, and subsequent perturbations of the hypothalamic-pituitary-thyroid axis.

1.4.2 Ecotoxicological Testing (Dr. Cardwell)

1. Will the additional ecotoxicological studies currently underway be sufficient to characterize the ecotoxicological potential of ammonium perchlorate and the perchlorate ion? If not, explain what data needs will be unmet and describe further studies that should be considered, present the rationale for the studies, and provide overviews of the types of experimental designs that will be needed.

Appendix F

Copy of Written Comments Received by RTI from Outside Observers Before the Workshop

Toxicology Excellence for Risk Assessment

TERA *a nonprofit corporation dedicated to the best use of toxicity data for risk values*

February 1, 1999

Ms. Susan Goldfarb
Research Triangle Institute
P.O. Box 12194
Research Triangle Park, NC 27709-2914

Dear Ms. Goldfarb:

On behalf of the Perchlorate Study Group, *TERA* has reviewed EPA's document titled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information*. This letter transmits to the Peer Review Panel our comments on the scientific basis of the proposed reference dose. Overall, we wish to commend EPA on the quality of its review. Several issues may elicit differing scientific judgment, however, which warrant the consideration of the Peer Review Panel as it reviews the document.

Thank you for the opportunity to highlight these issues for the Peer Review Panel. If you need additional information, please feel free to contact me by phone at (513) 542-7475, or by email at dourson@tera.org.

Sincerely,

Michael L. Dourson

Michael L. Dourson, Ph.D, DABT
Director
Toxicology Excellence for Risk Assessment

cc: M. Girard, PSG

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Comments on EPA's Choice of Critical Study and Data Set[1]

EPA has identified disturbance in thyroid homeostasis as the critical effect for perchlorate, and selected incidence of thyroid hypertrophy in pups culled on postnatal day 5 (PND 5) from the neurobehavioral developmental study (Argus 1998) as the data set that best represents the critical effect. However, we ask several questions regarding the reliability, validity, and use of these data and feel that answers are needed before these data are used as the basis of a RfD.

1. Was this part of the study designed properly to detect effects caused by *in utero* exposure?
2. Was the correct data and statistical analysis selected for the evaluation of PND 5 pups?
3. Were appropriate data used in the benchmark modeling that was used to confirm the dose level for the critical effect?

As EPA notes on page 6-12 of its report, any effects observed in PND 5 pups are likely to have been the result of *in utero* exposure. Since the effect in this case actually occurs in the female exposed to the chemical, the appropriate measure of response is the litter, not the individual offspring. While the main study design for the neurobehavioral developmental study evaluated one pup/sex/litter to control for litter effects, the histopathological analysis of the culled pups did not follow this design. All thyroids were collected by Argus Laboratories at culling, fixed in 10% neutral buffered formalin, and shipped to the Sponsor (AFRL/HEST).

Moreover, six thyroids per sex/dose group were selected for examination; selection of thyroids was accomplished by randomizing the samples from each treatment group using a computer program for randomization. Separate randomization was done for selection of males and females. First, it should be noted that there were 25 dams/group in the study, but thyroid histopathology from only 6 pups/sex/group was evaluated. This means that, for a given sex, less than 25% of the litters were sampled. EPA pooled the male and female data, improving the sampling of litters. However, in each dose group, between 30 and 60% of the combined male and female pups were littermates (York, personal communication). EPA appears to have taken the littermates into account by analyzing the data on a "per litter" basis. This meant that when a given litter was represented by *both* a male and a female, the average severity score of the male and the female were used as the litter score (Geller, USEPA, personal communication). Given the small sample size from each litter (1-2 animals/litter), is this an appropriate analysis method? Furthermore, given the small number of litters represented, is this analysis appropriate? We suggest that the panel evaluate whether enough litters were sampled to detect an effect caused by *in utero* exposure. In addition, we suggest that the panel consider what would be an adequate study design to address these issues.

As to the second question, in Section 5.2.3.2, EPA evaluates the thyroid histopathology results from the neurobehavioral developmental study. EPA notes (page 5-27, line 29-30) that the measurement of follicular epithelium cell height is a more sensitive indicator of thyroid effects than follicle diameter. In Appendix O of the Argus study report, Dr. William Baker describes two histopathology analyses of changes in cell height. In the first analysis, Dr. Baker conducted a subjective analysis of changes in cell height, ranking changes on a scale of 0 to 4. This is described in Appendix O (Results I) as follicular cell hypertrophy. In the second analysis

[1] All references are as per EPA Report or otherwise footnoted.

(reported in Appendix O under Results II), Dr. Baker quantitatively measured the height of follicular epithelium cells. Five follicles from each of three sections were measured. Results of the first (subjective) analysis indicate that there was no statistically significant increased incidence or severity of increased cell height in females. In males, there was a statistically significant increase in severity only at 10 mg/kg/day. Statistically significant increases in the number of males with follicular cell hypertrophy (i.e., subjective scoring of increased epithelial cell height) were observed at all doses. As reported in a personal communication from Dr. Baker, the follicular cell hypertrophy was subjectively scored as 0 (no change), 1 (minimal changes in general height), or 2 (mild changes in general height). No animals received a score of 3 (moderate), which would have included any increase in cell number or cell division. The incidence of severity scores for increased epithelial cell height in males, as provided by Dr. Baker (personal communication) is shown in Table 1.

Table 1. Severity scores for increased epithelial cell height from PND5 pups

Severity Score	0 mg/kg-day	0.1 mg/kg-day	1.0 mg/kg-day	3.0 mg/kg-day	10.0 mg/kg-day
Males					
0	5/6	1/6	2/6	1/6	0/6
1	1/6	4/6	3/6	4/6	2/6
2	0/6	1/6	1/6	1/6	4/6
Females					
0	4/6	3/6	1/6	3/6	0/6
1	1/6	2/6	2/6	2/6	5/6
2	1/6	1/6	3/6	1/6	1/6

This table shows that the incidence of males with either no or minimal changes in cell height (severity scores 0 or 1) remains essentially the same until the 10 mg/kg-day dose group. The incidence of animals with mild increased cell height (severity 2) also does not change until the 10 mg/kg-day dose group. Consistent with the results of the subjective analysis that there is no clear effect until 10 mg/kg/day, statistically significant increases in the quantitative measurement of follicular cell height were observed only in the 10 mg/kg-day dose group. (This interpretation is in contrast to EPA's interpretation of a disparity between the subjective and quantitative evaluations.) As was observed in the subjective assessment, there was no statistically significant effect on measured cell height in females. Therefore, should the panel conclude that the PND 5 pup data is valid and reliable enough to be used in the risk assessment, we suggest that the panel evaluate whether the histopathology data from these animals has been appropriately evaluated and whether the quantitative measurement of follicular height should be considered a more accurate indicator of changes in thyroid histopathology.

As to the third question, EPA used a benchmark dose analysis of the data from the subjective assessment in order to confirm that a LOAEL of 0.1 mg/kg-day was appropriate EPA evaluated and modeled the frequency of occurrence of severity ratings of 0.5 and up. Given that severity 1 was classified as "minimal," should average severity ratings of 0.5 or 1 be considered an appropriate basis for risk assessment, or should only severities of 2 or greater be considered? The study pathologist noted in a personal communication that hyperplasia would have been graded severity 3 ("moderate") or higher, if it had been seen, while hypertrophy would have been graded 1 or 2.

Comments on EPA's Choice of Uncertainty and Modifying Factors for the Perchlorate RfD

Human Variability (H) [Default Value is 10-Fold; EPA's choice is 3-Fold]

Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences among humans.

EPA chose a value of 3 for this factor (obtained by reducing the toxicodynamic factor to 1), because the animal model used is for a fetal effect and the adult human hormone homeostasis is likely to be more stable. We agree with EPA, and add that the use of this factor will also likely protect sensitive adult subgroups such as Graves' patients and people who are deficient in iodine intake (*TERA*, 1997). EPA's description of the overlap between this factor with the factor used for LOAEL to NOAEL is conceptually correct, but difficult to communicate. We suggest that the distinction be maintained between these two factors. We encourage EPA to review data on the current pharmaceutical use of perchlorate, and, if appropriate reevaluate the dose-response relationship from existing human studies as described in EPA's report (Chapter 3). Such an analysis was requested at a previous peer review meeting (*TERA*, 1998a). Moreover, two occupation studies are now available (Gibbs et al., 1998; Lamm et al., 1999[2]) which should be gleaned for any relevant information in this reevaluation of dose-response relationship. Please see a separate section of this comment (on use of human data) for more details.

Inter-Species Variability (A) [Default Value is 10-Fold; EPA's choice is 3-Fold]

Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences between animals and humans.

EPA chose a value of 3 for this factor (obtained by reducing the toxicodynamic factor to 1), because rats are known to be more sensitive than humans to thyroid disturbance. We agree with EPA's initial choice, but encourage EPA to analyze existing comparative data on rats and humans to quantify the rat's greater sensitivity to the iodide blocking effects of perchlorate. Sufficient human and rat data now exist on which to make a tentative assessment of this sensitivity. For example, The data of Meyer (1998) found on page 5-62 of EPA's report, could be compared to the data of Burgi et al. (1974). Moreover, the report of Sterner and Mattie (1998)[3] gives information on the perchlorate discharge tests in humans and rats. These data should be compared as well. Such an analysis was requested at a previous peer review (*TERA*, 1998a).

Subchronic-to-Chronic Extrapolation (S) [Default Value is 10-Fold; EPA's choice is 1-Fold]

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[2] Lamm, S.H., L.E. Braverman, F. Xiao, K. Richman, S Pino and G. Howearth. 1999. Thyroid health status of ammonium perchlorate workers: A cross-sectional occupational health study. Accepted for publication in J Occup and Environ Medicine.

[3] Sterner, T.R. and D.R. Mattie. 1998. Perchlorate literature review and summary: Developmental effects, metabolism, receptor kinetics and pharmacological uses. AFRL-HE-WP-TR-1998-0106.

EPA chose a value of 1 for this factor, because the mode of action of perchlorate indicates that if the current critical effect is maintained then it will be protective of downstream events. EPA supported this choice of a 1-fold factor with the fact of the relatively short half-life of perchlorate in the body. We agree with EPA's choice of factor for the reasons stated.

<u>Insufficient Database (D) [Default Value is 10-Fold; EPA's choice is 3-Fold]</u>

EPA chose a value of 3 for this factor, because of the data outstanding from the 2-generation reproductive and immunotoxicity studies. We agree with EPA's choice, and also agree with EPA that appropriate data from these two studies, when finalized, will likely reduce the need for this factor in the future.

<u>LOAEL to NOAEL (L) Extrapolation [Default Value is 10-Fold; EPA's choice is 3-Fold]</u>

EPA chose a value of 3 for this factor for perchlorate, because the critical effect and its LOAEL represent minimal toxicity. In fact, as EPA suggests, an argument could be maintained that the critical effect represents a normal homeostatic response to the decrease in T3 & T4 and increase TSH, and should therefore be considered a NOAEL. As one way forward, we encourage EPA to ask experts in thyroid disturbance whether this effect should be consider adverse, or whether it represents part of the normal homeostatic response of the rat (see a later section of these comments for more details). If experts agree that EPA's suggested critical effect represents homeostasis, then a different effect could be selected, or this dose could be considered a NOAEL which would obviate the need for this factor.

<u>Modifying Factor (MF) [Default is 1-Fold; EPA's choice is 1-Fold]</u>

EPA considers a default value of 1 as appropriate for a modifying factor for perchlorate. This is because the outstanding uncertainties for perchlorate can be adequately addressed with the standard factors. We agree with EPA on the use of 1-fold for this factor.

<u>Composite Uncertainty and Modifying Factors [Each Data Base Has Its Own; EPA's Choice for Perchlorate is 100]</u>

EPA recommends an overall uncertainty factor of 100 for perchlorate. We agree with EPA's initial recommendation, but anticipate that the inclusion of the complete 2 generation reproductive and immunotoxicity studies in the perchlorate database will likely reduce the composite factor of 100 to 30-fold. This is because the data base uncertainty factor of 3 will likely not be necessary after the new studies are finished. Moreover, if thyroid experts suggest a different critical effect as the basis of the RfD, or if an analysis of existing comparative data on rats and humans yield a firm quantitative value, the issue of uncertainty factors may need to be revisited.

Comment of EPA's Use of Human Data in Hazard Characterization

Although the EPA assessment presents information on some of the available human data, these data do not greatly contribute to EPA's hazard characterization of perchlorate. We suggest that the available human data should be reanalyzed. This would lead to a better understanding of the perchlorate dose-response curve in humans, and to a clearer description of the relative sensitivity of rats and humans to perchlorate. Such an analysis was suggested at a previous peer review meeting (*TERA*, 1998a).

Several types of human data are available for consideration of the perchlorate RfD; some of these data are new. These studies include a small epidemiology study (Lamm Doemland; 1999[4]---new), occupational exposure of perchlorate workers (Gibb et al, 1998; Lamm et al., 1999---new)) experimental dosing of normal human volunteers Burgi et al, 1974; Brabant et al., 1992), and treatment of Graves' disease patients with perchlorate (section 3.1 of EPA report). Human studies are also available on use of perchlorate discharge tests in infants as a diagnostic tool (Sterner and Mattie, 1998---not cited in EPA report), which may prove to be very useful in the comparison with animal perchlorate discharge tests. Treatment of patients given the heart drug amioderone (Sterner and Mattie, 1998) are also available, but we feel that the use of these latter data to determine and RfD is problematic. We very briefly describe several of these studies below and in Table 2.

Lamm and Doemland (1999) conducted an analysis to determine whether the perchlorate-containing drinking water increases the risk of congenital hypothyroidism. Perchlorate was detected in the drinking water supplies of seven counties in California and Nevada at levels of 4-16 ug/L in 1997. The data from the neonatal screening programs of the state health departments were analyzed for incidence of congenital hypothyroidism in those counties in 1996-1997. Total 700,000 newborns were screened during this period. Two hundred and forty-nine cases were identified in these newborns while 243 were expected based on the state-wide incidence. The risk ratio is 1.02 with 95% confidence limits of 0.9-1.2. Studies such as this can assist the determination of whether an increase of perchlorate-induced thyroid problems is evident. They are much more limited in assisting in the determination of a RfD, however.

Gibbs et al. (1998) conducted a cross-section occupational epidemiology study to evaluate thyroid, liver, kidney, and bone marrow function of workers at an ammonium perchlorate production facility. The study examined the health effects due to either single-shift exposure or working lifetime exposure. A total of 101 workers participated in the single shift study. Among them, 18 workers were exposed to ammonium perchlorate at doses ranging from 0.0002 to 0.436 mg/kg-day with an average of 0.036 mg/kg-day, and 83 workers who were never exposed to perchlorate were used as controls. The thyroid function measured before and after the work shift by T4, T3 resin uptake, TSH and free T4 index was analyzed. The estimated exposure was not a significant predictor of the cross-shift change in any of the thyroid parameters. The only significant finding in this study was cross-shift TSH changes that were greater for those who worked 12-hour shifts than for those who worked 8-hour shifts.

[4] Lamm, S.H. and M. Doemland. 1999. Has perchlorate in drinking water increased the rate of congenital hypothyroidism? Submitted to J Occup and Environ Medicine.

In the working lifetime study (Gibbs et al. 1998), 66 workers in low dose group exposed to perchlorate at doses ranged from 0.5 to 7.0 mg/kg (mean = 3.5), and 108 workers in high dose group exposed to doses ranged from 8.0 to 88 mg/kg (mean = 38). One hundred and ninety-two workers who never exposed to perchlorate were used as controls. Duration of these workers' exposure ranged from 1 to 27 years with an average of 8.3 years. The thyroid functional parameters as mentioned above were analyzed. Additionally, standard clinical blood test parameters of liver, kidney, and bone marrow function were evaluated to determine effects of chronic perchlorate exposure on these organs. The study shows that chronic perchlorate exposure did not have effects on thyroid, liver, kidney, or bone marrow functions.

EPA quite correctly pointed out several difficulties in the use of the Gibbs et al. (1998) study for risk assessment. The recent study of Lamm et al. (1999), however, may help resolve some of these difficulties and we feel that EPA should reconsider the use of the Gibbs et al work.

Moreover, Lamm et al. (1999) conducted a cross-sectional study to investigate thyroid health status of ammonium perchlorate workers. Thirty-seven employees from an ammonium perchlorate production plant and 21 from a sodium azide production plant (served as a control) participated in this study. The exposure workers was divided into 3 groups with average exposure levels at 0.33, 6.5, and 59 mg/day perchlorate in total airborne particulates while control group exposed to only 0.01 mg/day. Urinary perchlorate measurements indicated that workers in the exposed groups absorbed 4, 11 and 34 mg perchlorate per day while control workers absorbed 1 mg perchlorate. Thyroid function was evaluated based on clinical examinations and measurements of serum TSH, FTI, T4, thyroid hormone binding ratio (THBR), or TPO antibodies. No difference in the thyroid function was observed within the four groups. In addition, there was no evidence of hematotoxicity measured as blood cell count in these groups. Thus, this study found no evidence of an adverse effect of perchlorate exposure on thyroid status among workers. We feel that EPA should consider this study in the determination of an RfD.

EPA describes the following three studies, but did not use them directly in the determination of an RfD. Stanbury and Wyngaarden (1952) evaluated perchlorate in patients with Graves disease and found that perchlorate caused the discharge of iodine accumulated in the thyroid and blocked the uptake of iodine into the thyroid. Burgi et al. (1974) examined the effects of perchlorate on the secretion of endogenous iodine by the normal human thyroid gland. Five healthy volunteers received tracers of I^{125}-iodide and I^{131}-thyroxine for 17 days followed by 600 mg/day perchlorate (9.7 mg/kg/day, based on actual reported average body weight of 61.8 kg) perchlorate for 8 days. Brabant (1992) administered potassium perchlorate to healthy volunteers as a means to study changes in TSH concentration and release in response to a decrease in iodine supply to the thyroid. During the first 4 weeks of the study, the volunteers were given 200 ug/day iodine. After iodine supplementation was discontinued, the volunteers were orally administered 900 mg/day of potassium perchlorate for four weeks to induce a state of iodine depletion. At the end of the 4-week perchlorate treatment, levels of thyroid hormones were measured.

These 3 studies by themselves are not strong enough to form the basis of a RfD (*TERA*, 1998a). However, they may help contribute to an understanding of the potential dose response curve in humans in conjunction with the occupational studies shown above. Furthermore, we believe that data from these three studies might be compared with data from Meyer (1998) or Alterwill et al. (1987) in rats to ascertain whether an uncertainty factor of 3 for toxicokinetics should be replaced with data. If the human and rat data are not strictly comparable, then a generalization might be possible to allow a different choice of uncertainty factor between rats and humans.

Sterner and Mattie (1998) also describe a series of studies on perchlorate discharge tests in humans and rats. The information in humans encompasses a number of conditions and includes children. EPA may wish to compare some of these studies to the information found in Meyers (1998) and Atterwill et al. (1987). Such a comparison may allow the use of a different uncertainty factor for animal to human variability.

Comment on Evaluation of Thyroid Homeostasis and Choice of Critical Effect

EPA has proposed a model for perchlorate mode of action that shows a sequential progression of effects (Figure 6-1). However, maintenance of normal thyroid function is the result of a series of feedback mechanisms, and adverse effects develop after the normal homeostatic capability of the thyroid has been exceeded. An alternative model is proposed in Figure 1. No change in a single thyroid parameter is indicative of adverse thyroid effects. Rather the degree of change, and the interaction of all different thyroid effects, including hormone level alterations, thyroid histopathology, and thyroid weights, should be evaluated to identify the perchlorate dose which results in animals moving from normal homeostasis into an altered function. Therefore we suggest that the panel establish criteria for distinguishing thyroid homeostasis from thyroid adversity. Some suggested criteria include focusing on changes in T4 as reflective of thyroid activity more than T3 because many other factors affect T3, and focusing on thyroid histopathology only when it is accompanied by changes in TSH at the same dose levels (Capen, personal communication).

For example, in all of the studies evaluated, EPA has designated statistically significant changes in hormone levels as LOAELs. However, since thyroid hormone levels can be widely variable due to homeostasis, it is important to evaluate the biological significance of altered hormone levels in addition to statistical significance. A slight, but statistically significant, decrease in hormone levels could have no biological consequence at all. Therefore, it is important to compare the dose levels that result in slight hormone changes with those that demonstrate alterations to the thyroid gland. Looking at the database as a whole, a common pattern is observed (e.g., see Figure EPA 5-9). Statistically significant changes in hormone levels occur at the lower doses, but then hormone levels remain constant over a range of doses up to 100-fold higher. At doses higher than 1 mg/kg-day hormone levels change again and at this point, thyroid histopathology and increased thyroid weight is observed. This pattern suggests that between the doses of 0.01 and 1 mg/kg-day, perchlorate is having an effect, but within this dose range the thyroid is able to maintain normal homeostasis. Conversely, alterations to the thyroid gland in the absence of hormone changes are not likely to be due to perchlorate exposure. In the neurobehavioral developmental study, hypertrophy/hyperplasia is observed in the pups at postnatal day 5 at the lowest dose (0.1 mg/kg-day). However, T3 was not statistically decreased in these animals until 0.3 mg/kg-day, T4 was not statistically decreased until 3 mg/kg-day, and

TSH was not statistically increased until 10 mg/kg-day. Are the effects on thyroid histopathology likely to be the result of altered thyroid hormones?

Table 2. Selection of Available Human Studies Ordered (Roughly) by Increasing Dose.

Study	Subject	Dose (mg/kg-day)	Duration	Response	Comments
Lamm and Doemland 1999	Newborn babies (n=700,000)	0.004-0.016 mg/L in drinking water (0.00011-0.00046)[a]	Pregnancy	No increase in incidence of congenital hypothyroidism	Incidence analysis
Gibbs et al. 1998	Perchlorate workers *Single shift study* (n=83 in control group, n=18 in exposed group)	0.036	One work shift (8 or 12 hours)	Greater cross-shift TSH changes in 12-hour shift workers than 8-hour shift workers. No change in other thyroid functions	Cross-sectional study
	Lifetime study (n=192 in control group, n=174 in exposed group)	Low cum. dose avg.=3.5 mg/kg, high cum. dose avg.=38 mg/kg	Avg. exposure =8.3 years	No significant effect on thyroid, bone marrow, liver, or kidney function	
Lamm et al. 1999	Perchlorate workers (n=21 in control group, n=37 in exposed group)	0.01-59 mg/day in airborne particulate (absorbed doses of 0.014-0.486)[b]	12 h/day, 3 days/week for >5 years	No changes in thyroid function and clinical examination	Cross-sectional study
Stanbury and Wynyaarden 1952	Graves' disease patients (n=8)	3 mg -500 mg $KClO_4$ (0.04 - 7.14)[b]	Once	*Thyroid I release:* 10 mg: partial (40-45%) decrease of thyroid ^{131}I count; 100 mg: ^{131}I count decreased to control level (measured at thigh); *Thyroid I uptake inhibition:* 100 mg: inhibited ^{131}I accumulation	Measure radioactivity (I^{131}) in thyroid
Burgi et al. 1974	Healthy humans (n=5)	3 x 200 mg perchlorate (9.7)[c]	8 days	Increase in non T_4 bound I secretion from 40 ug/24 h (control) to 66 ug/4 h (perchlorate)	Double labeling (^{125}I and ^{131}I) and measure radioactivity in blood.
Branbant et al. 1992	Healthy humans (n=5)	3 x 300 mg (12.9)[b]	4 weeks	Decrease thyroid I from 4 mmol/ml (before treatment) to 3.0 mmol/ml (after perchlorate); decrease in blood TSH and increase in blood thyroglobulin	Measure thyroid (radioactivity) Measure blood hormone

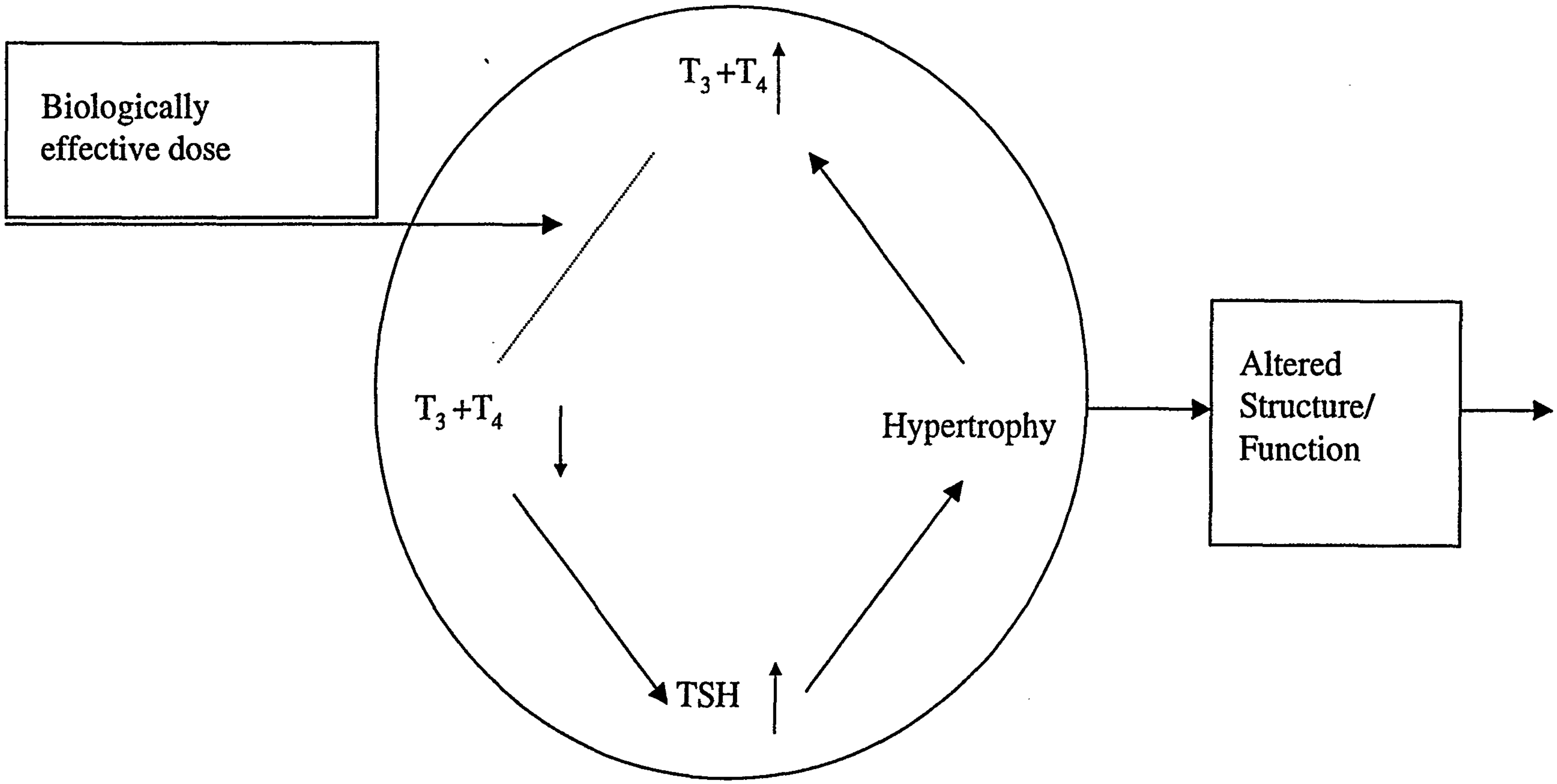
Estimated value(s) based on:

a. default 2 liter water consumption per day and adult body weight of 70 kg;

b. a default body weight of 70 kg for an adult;

c. the average body weight of 61.8kg reported by authors;

Early Bio[illegible]cal Homeostasis



Gay Goodman
Intertox

INTERTOX

Comments on the December 31, 1998, External Review Draft of the EPA/NCEA Document, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information"

Submitted to the External Peer-Reviewers

Gay Goodman, Ph.D., D.A.B.T.

February 1, 1999

I. Introduction

The External Review Draft[1] describes the derivation of a revised RfD for perchlorate based on animal data. In Section II of this commentary, the derivation of this revised RfD is critiqued and suggestions are offered for a more physiologically integrated approach to using the animal data as the basis of the RfD.

The RfD derivation presented in the External Review Draft is essentially devoid of reference to available information on safe levels of human exposure to perchlorate. More to the point, additional human data are currently being gathered; these address the effects of short-term perchlorate exposures on thyroidal uptake of iodide, serum levels of thyroid hormones, and the kinetics of perchlorate elimination. Preliminary results are expected within the month, and final results a few months later. Section III briefly suggests how the human data could be utilized to ensure greater relevance to humans in the derivation of a revised RfD.

II. Animal Data As the Basis of the RfD

A. Choice of the Critical Study and Critical Endpoint

In the External Review Draft, EPA/NCEA evaluated the results of six new, animal-toxicity bioassays. Final study reports were available for four of these (Caldwell *et al.*'s 14-day study in rats, Springborn Laboratories' 14-day and 90-day study in rats, a neurodevelopmental study in rats, and a developmental toxicity study in rabbits), while only incomplete study results were available for the other two (a two-generation reproductive study in rats and an immunotoxicity study in mice). Based upon the results of the new studies, including some reanalyses of raw data by EPA/NCEA, critical effects were chosen and a principal study was identified. According to the External Review Draft (p. 6-2), "the overwhelming weight of the evidence from these studies support[s] the use of the hormone and thyroid histology evidence as the choice for critical effects." The critical study chosen was the neurodevelopmental study. The dose-response for follicular hypertrophy and decreased lumen size in postnatal-day-5 (PND5) pups (as revealed by standard histopathology techniques) formed the basis of the RfD derivation. To quantify the dose-response for these endpoints, EPA/NCEA performed contingency-table analyses that allowed severity and incidence to be considered together. For both sexes combined, EPA/NCEA found that the lowest dose tested, 0.1 mg/kg-day, produced a statistically significant increase in the incidence and severity of follicular-cell hypertrophy (*i.e.*, increased cellular height and/or diameter) and decreased size of the follicular-cell lumen. Based on these results, the lowest-observed-adverse-effect level (LOAEL) for the critical effect in the principal study was determined to be 0.1 mg/kg-day.

EPA/NCEA has made a good case for focusing on the rat neurodevelopment study, particularly the PND5 data, above all other animal data considered. The new animal bioassays support the premise that the thyroid is the most sensitive target organ. Furthermore, rats exposed to perchlorate *in utero* are, as expected, more susceptible to changes in follicular-cell morphology than are weanling rats exposed for 14 or 90 days. The question remains, however, whether such changes are adverse in and of themselves. Are these changes merely histologically identifiable or are they indicative of a pathological process?

[1] "External Review Draft" is used herein as an abbreviation for the EPA/NCEA/ORD document, "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information."

B. Physiological Significance of Follicular Hypertrophy and Decreased Lumen Size

EPA/NCEA has argued, persuasively, that an exposure which produces transient effects of no toxicological significance to the adult animal might, on the other hand, produce permanent damage to the fetus or neonate. If exposure to a substance at a given dose produces irreversible histopathological changes, then it would be reasonable to conclude that a toxic threshold has been exceeded. However, no evidence for a permanent lesion has been found in rat pups exposed *in utero* and *via* breast milk to a maternal perchlorate dose as high as 10 mg/kg-day. The increased incidence of follicular-cell hypertrophy observed on PND5 in the 0.1 and 1.0 mg/kg-day dose groups was no longer evident in pups sacrificed on PND10. In pups with dosing discontinued on PND10, levels measured on PND22 were elevated relative to control levels only in the 1.0 mg/kg-day group; however, the statistical significance was not given and the absence of an effect in the 3.0 and 10 mg/kg-day groups argues against the relevance of this elevation. Table 1 shows the incidence data as given in Tables 5-3 (p. 5-29) and 5-4 (p. 5-30) of the External Review Draft.

EPA/NCEA has classified increased follicular-cell size (hypertrophy) and decreased follicular-cell lumen size as adverse effects. Indeed, there might be circumstances when the histological findings in question are corollary to an underlying pathology. But EPA/NCEA has not provided convincing evidence that such cellular changes, in and of themselves, fall outside the realm of physiologically normal thyroid morphology.

Table 1. Incidence Ratio of Any Evidence of Follicular-Epithelial-Cell Hypertrophy Among Rat Pups in the 1998 Neurodevelopmental Study of Perchlorate by Argus Laboratories, as Determined by Standard Histology

		Perchlorate Dose to the Dams (mg/kg-day)			
Time of Sacrifice	Control	0.10	1.0	3.0	10
PND5	0.25 (3/12)	0.67 (8/12)	0.75 (9/12)	0.67 (8/12)	1.00 (12/12)
PND10	0.40[a]	0.40[a]	0.40[a]	1.00[a]	1.00[a]
PND22[b]	0.52[a]	0.48[a]	0.68[a]	0.52[a]	0.48[a]

[a] The External Review Draft did not provide the absolute number of animals examined.

[b] Perchlorate exposure discontinued on PND10.

C. Correlation Analyses (Figures 6-3 to 6-16 of the External Review Draft)

In the External Review Draft, EPA develops a toxicologic mode-of-action model "proposed to map the relationships between external dose, internal dose, the biologically effective dose, and altered structural and functional parameters of established relevance to risk assessment" (p. 6-10.) Asserting that "the earliest biological effect, changes in thyroid and pituitary hormones" is the precursor for potential adverse outcomes, EPA notes, "The difficulty in designating an effect level for these perturbations, however, was in the degree of change to designate as adverse." Thyroid hormone/thyroid histology correlation analyses were used to "further support the mode-of-action mapping" model (p. 6-12). The analyses consist of the paired comparisons outlined in Table 2. EPA's hypothesis is that positive correlations for T3 *vs.* T4 and negative correlations for T3 (or T4) *vs.* TSH "are expected if these perturbations are affecting thyroid economy." The hypothesis is extended further: "Positive correlations between TSH and thyroid histopathology are expected, whereas T3 or T4 would be correlated negatively (inversely) with thyroid histopathology." Note that the use of the word "histopathology" in this context suggests the unwarranted assumption that the observed histological changes are indeed pathological in nature.

There is no doubt that, in most cases, the analyses revealed statistically robust correlations of the expected sign. The question is whether the results were skewed in favor of the relationships corresponding to higher perchlorate doses. In other words, would the same correlations be found if only the parameter values corresponding to perchlorate doses of, say, 1 mg/kg-day and below were included in the analyses? In the absence of access to the original data, much can be gleaned by visual inspection of figures 6-3 to 6-16. For T3 *vs.* T4, a positive correlation seems to be present even at the lower perchlorate doses. For T4 *vs.* TSH, there is evidence for and against a negative correlation. For the rank order of T4 or TSH *vs.* histopathological severity, it is doubtful that any statistically meaningful correlation would be found at lower doses.

- In Caldwell *et al.*'s 14-day rat study, even if doses above 1.1 mg/kg-day are ignored, an inverse relationship between T4 and TSH is evident upon visual inspection of Fig. 6-3 (p. 6-15). However, this finding contradicts the results of Springborn Laboratories for rats exposed likewise for 14 days (Fig. 6-8, p. 6-20) or of Argus Laboratories for PND5 rats following exposure *in utero* and *via* breast milk (Fig. 6-12, p. 6-24). In the latter two studies, removal of the 10 mg/kg-day data (leaving 1.0 mg/kg-day as the highest dose) would yield a *positive* relationship between T4 and TSH, *i.e.*, opposite to what the EPA/NCEA model predicts. For the remaining analyses of T4 *vs.* TSH (Figs. 6-6, 6-10, and 6-15, pp. 6-18, 6-22, and 6-27), visual inspection of the figures is insufficient to allow firm prediction of the consequences of removal of the data corresponding to perchlorate doses above 1 mg/kg-day; for these analyses, direct analysis of the raw data would be required.
- With respect to the analyses of T4 or TSH rank-order *vs.* severity classification for hypertrophy or decreased lumen size (as determined by standard histopathology), visual inspection of Caldwell *et al.*'s 14-day rat data (Figs. 6-4 and 6-5, pp. 6-16 and 6-17) does not allow prediction of the consequences of removal of the data for higher perchlorate doses. However, visual inspection of Springborn Laboratories' 14-day/90-day combined (Fig. 6-7, p. 6-19) or 14-day (Fig. 6-9, p. 6-21) data in rats indicates that, in both cases, the correlations are clearly dependent on the high-dose group (100 or 10 mg/kg-day). Thus, over perchlorate dose ranges of 0 to 30 mg/kg-day for the 14-day/90-day combined data and 0 to 1 mg/kg-day for the 14-day data there appears to be no correlation of the severity of hypertrophy or decreased lumen size with the rank order of either T4 or TSH. To definitely confirm (or disprove) this assertion, one would have to perform sequential statistical analyses: removing the top dose, then the next highest dose, and so on, to see what remains of the relationship between T4 or TSH rank order and follicular-cell morphology after each round of data removal. Performance of this task would require access to the raw data.

If there is no correlation between the thyroid hormones T4 or TSH and thyroid histopathology (follicular hypertrophy or decreased lumen size) at perchlorate doses of 1.0 mg/kg-day and below, it is not useful to argue that such correlations support a model in which thyroid hormone changes *of any magnitude* are considered to be potentially causal to histological changes. It is far more likely that, even in an animal as sensitive as the rat, some fluctuations in T3, T4, and TSH are tolerated without consequent alteration of follicular-cell morphology.

D. Historical Control Data on Serum T3 and T4 in Rats

In the External Review Draft, EPA/NCEA examines the relationship between perchlorate dose and thyroid hormone levels. Although statistical analysis of the dose-response is straightforward, lingering questions remain concerning the physiological significance of small, statistically significant changes. One means of addressing such concerns is to examine the historical data base for thyroid hormones in the control groups of animal bioassays. If a given dose of perchlorate produces a change that falls within the normal range of thyroid-hormone levels seen in animal-bioassay control groups, it would seem unreasonable to argue that such a response is part of a continuum leading to adverse effects.

Using data from bioassays sponsored by the National Toxicology Program (NTP), Dr. Greg Travlos of the National Institutes of Environmental Health Sciences (NIEHS) has assembled a data base of blood-chemistry parameter values found in control animals at the 13-week sacrifice. After excluding data considered by Dr. Travlos to be invalid or unreliable, there were data from thirteen bioassays for T3 and sixteen for T4. At this time, the entire data base on TSH is considered to be unreliable, either because of standardization issues, measurement errors, reporting errors, or some combination thereof (Dr. Greg Travlos, personal communication).

Figures 1 and 2 present mean values of serum total T3 and T4 in control-group rats at the 13-week termination point in NTP-sponsored studies. Also shown in Figures 1 and 2 are serum total T3 and T4 levels measured in the 90-day, drinking-water exposure study of perchlorate in rats. Note that mean serum T3 levels were higher in both male and female perchlorate controls than in any of the NTP control groups. Mean serum T4 levels in the perchlorate controls of both sexes were in the middle of the range defined by the NTP control groups. Up to the highest perchlorate dose tested, 10 mg/kg-day, serum T3 and T4 levels were well within the ranges defined by the means of the NTP control groups.

Possible inter-strain differences in T3 levels cannot be excluded, although any such differences are unlikely to be of consequence. With respect to T4 levels, the available data do not support the hypothesis that inter-strain differences in variability are significant. Sprague-Dawley rats were used in the 90-day, drinking-water exposure study of perchlorate, while Fisher-344 (F344) rats were used in all NTP studies reporting T3 levels and in fourteen of the studies reporting T4 levels; Sprague-Dawley rats were used in the other two. The latter two studies reported mean serum T4 levels of 3.7 and 3.9 μg/dl in males and 4.7 and 5.0 μg/dl in females. T4 levels in the NTP Sprague-Dawley males fell at the low end of the NTP range, in between the levels observed for the 1 and 10 mg/kg-day perchlorate groups. By contrast, T4 levels in the NTP Sprague-Dawley females were near the middle of the NTP range, above the level observed in the zero-dose perchlorate group. These observations on the two NTP Sprague-Dawley control groups suggest that this rat strain is likely to demonstrate patterns of T3 and T4 variability similar to those observed for the larger, F344 rat-dominated NTP control data base.

In summary, the historical data base provides evidence *against* the assumption that in rats treated for 90 days with drinking water containing perchlorate at levels as high as 10 mg/kg-day, the resultant depression in T3 and T4 should be considered as physiologically abnormal or adverse.

E. Motor Activity Measurement in the Neurodevelopmental Study

It should be kept in mind that, as indicated on p. 5-37 of the External Review Draft, the non-significant increase in motor activity on PND14 in the pups of dams receiving the lowest-dose, 0.1 mg/kg-day, occurred "in only one gender on only 1 day out of 4 test days" and that "the effect seen in the males on PND14 may indeed be a Type I error and would not be found again if this experiment was repeated." It is reasonable for EPA/NCEA to request that the trial be performed again, but the lack of dose-response observed among the 0, 0.1 and 1.0 mg/kg-day groups indicates that the most likely outcome is no effect of treatment at doses below 3 mg/kg-day.

F. Recommendations for a Critical NOAEL Based on the Animal Data

1. Increased Size of the Corpus Callosum on PND12

Although the thyroid is certainly the principal target organ for the actions of perchlorate, it is perhaps a mistake to conclude that the critical effect can be found by looking at the thyroid or thyroid hormones directly. The thyroid apparently functions quite well (*i.e.*, in an adaptive fashion) in weanling rats given perchlorate for 90 days at doses up to 10 mg/kg-day, the highest dose tested; this is true also for the PND5 pups of rat dams given perchlorate at 10 mg/kg-day and below. In the latter (neurodevelopmental) study, recovery from increased thyroid follicular-cell hypertrophy in the 10 mg/kg-day group occurred by PND22 when perchlorate treatment was stopped on PND10 (see Table 1, above). From Figs. 5-11 and 5-12 of the External Review Draft (pp. 5-33 and 5-34) one can see that in PND5 rats, T3 and T4 were decreased approximately 60% and 30%, respectively, at a perchlorate dose of 3 mg/kg-day, while TSH was increased approximately 20% at a perchlorate dose of 10 mg/kg-day (Fig. 5-13, p. 5-35). It is conceivable that some developing organs might be affected by thyroid hormone changes of this magnitude during development. For example, it is possible that the 27% increase in the size of the corpus callosum in males and females combined (observed on PND12 in the pups of rats given perchlorate at 10 mg/kg-day) is secondary to altered thyroid hormone levels. Although the significance of this finding is unclear, it seems reasonable that "EPA considers a 27% increase in the size of any brain region to be a potentially adverse effect [ref.]" (p. 5-26). The LOAEL and NOAEL derived by EPA/NCEA from this study, 10 mg/kg-day and 3 mg/kg-day, respectively, constitute an appropriate departure for deriving an RfD.

Note, however, that Argus Laboratories' neurodevelopmental study is a poor model for the effects of chronic perchlorate administration on the developing fetus. Perchlorate exposure began on GD0, thus ensuring that the rat dams (and thus their embryonic fetuses) experienced the shock of shifting serum levels of thyroidal hormones at a very critical time. If the dams had begun perchlorate treatment several weeks prior to mating, it seems likely that the changes in thyroid hormone levels, thyroid histology, and corpus-callosum size observed on PND5 would have been significantly muted, perhaps to the point of insignificance. In this context, it is interesting that one study has found that the fetal rat brain is able to maintain T3 homeostasis to a greater extent than other fetal tissues under the stress of variations in the maternal supply of iodine, T3, or T4 (Morreale de Escobar *et al.*, 1992).

2. Immunotoxicity Results in Mice

The results of the ongoing immunotoxicity study should be evaluated carefully before proceeding with the derivation of a new RfD. The results on macrophage phagocytosis are too inconsistent and too transitory to form the basis of an RfD. According to the External Review Draft, results from a test of humoral immunity (antibody response to SRBC antigen) are anticipated by the date of the peer-review workshop, while repeats of several tests of host resistance (to *L. monocytogenes* and B6F10 tumor cells) are not expected to be complete before June 1999.

III. Utilizing Human Data and Rat/Human Comparisons in the RfD Derivation

A. Ongoing Exposure Studies

One flaw of the External Review Draft is its failure to allow for existing health effects data gathered in occupational and clinical studies of perchlorate exposures. A more important flaw is that the revised RfD was derived during the time that two pertinent exposure studies were in the planning stages (or had been initiated). The Air Force Research Laboratory (AFRL) is currently conducting single-dose and 14-day exposure studies of the kinetics of perchlorate inhibition of thyroidal iodide uptake in rats. Dr. Lewis Braverman of Brigham and Women's Hospital, a well-known expert on the human thyroid, is in the process of conducting an exposure study in human volunteers. The Braverman study is examining thyroidal iodide uptake, serum levels of thyroid hormones, and the elimination kinetics of perchlorate at doses considerably lower than those hitherto tested in humans. Because perchlorate appears to exhibit nonlinear elimination kinetics, it is important to obtain animal and human toxicity health-effects data at doses as close as possible to the environmentally relevant range of exposures (Goodman, 1998). It is also important to be able to compare the inhibition of iodide uptake and the kinetics of perchlorate elimination in rats with those in humans. The data gathered by AFRL and Dr. Braverman should facilitate this comparison. The AFRL studies in rats and the Braverman study in humans are expected to conclude their data-gathering phases in February. The scientific basis of the revised RfD for perchlorate would be greatly strengthened by delaying the risk assessment process until the data from these studies can be taken into consideration.

B. Thyroid Homeostasis

It is well known that humans are able to maintain normal thyroid function over a wide range of iodine intake. This manifests, for example, in a narrower distribution of T4 values in normal humans than in control rats. In a study of 115 nongoitrous, Greek subjects exhibiting a broad spectrum of iodine intakes (*i.e.*, with urinary I/creatinine ratios varying from <50 to >250 μg/g), nearly all serum T4 values were in the 110 to 130 nM range (Moulopoulos *et al.*, 1988). This yields an estimated human T4 variation of approximately 15%, which is to be compared with the two- to three-fold variation in T4 for control rats in 13-week NTP studies (Figure 2). Simply put, the rat is expected to be more sensitive than the human to fluctuations in dietary iodine and to agents which affect thyroid hormone levels. EPA/NCEA would do well to explore these established differences before deciding on the appropriate uncertainty factors to use in deriving a revised RfD from a rat LOAEL or NOAEL.

IV. References Not Cited in the External Review Draft

Goodman, G. 1998. "Perchlorate Pharmacokinetics: Critical Review and Evaluation of Relevance to Low-Level Exposures in Humans." September 1, 1998. Submitted to EPA/NCEA.

Morreale de Escobar, G., Calvo, R., Obregon, MJ, Escobar del Rey, F. 1992. Homeostasis of brain T3 in rat fetuses and their mothers: Effects of thyroid status and iodine deficiency. *Acta Med. Austriaca* Suppl. 1: 110-116.

Moulopoulos, D.S., Koutras, D.A., Mantzos, J., *et al.* 1988. *J. Clin. Invest.* 11: 437-439.

Table 2. Paired Comparisons of Thyroid Hormone and Thyroid Histopathology Data as Reported in Figures 6-3 to 6-16 of the External Review Draft

Figure	Comparison	Data Description/Citation (as given in the External Review Draft)	Perchlorate Doses (mg/kg-day)
6-3	T3 *vs.* T4; T4 *vs.* TSH	14-day rat Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-4	T4 rank order *vs.* severity rating for follicular hypertrophy; T4 rank order *vs.* severity rating for decreased follicular lumen size	14-day rat study Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-5	TSH rank order *vs.* severity rating for follicular hypertrophy; TSH rank order *vs.* severity rating for decreased follicular lumen size	14-day rat Caldwell *et al.* (1995); Channel (1998a); Crofton (1998a)	0, 0.11, 0.25, 1.1, 2.6, 4.6, 11.5, 22.5
6-6	T3 *vs.* T4; T4 *vs.* TSH	14-day and 90-day rat, combined data Springborn Laboratories, Inc. (1998)	0, 0.01, 1.0, 10, 30, 100
6-7	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	14-day and 90-day rat, combined data Springborn Laboratories, Inc. (1998)	0, 0.01, 1.0, 10, 30, 100
6-8	T3 *vs.* T4; T4 *vs.* TSH	14-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-9	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	14-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-10	T3 *vs.* T4; T4 *vs.* TSH	90-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-11	T4, TSH rank order *vs.* severity rating for hypertrophy/ hyperplasia	90-day rat Springborn Laboratories, Inc. (1998)	0, 0.01, 0.05, 0.2, 1.0, 10
6-12	T3 *vs.* T4; T4 *vs.* TSH	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998a); York (1998c); Channel (1998b); Crofton (1998f)	0, 0.1, 1.0, 3.0, 10
6-13	T4 rank order *vs.* severity rating for follicular hypertrophy; T4 rank order *vs.* severity rating for decreased follicular lumen size	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998b); York (1998c); Channel (1998b); Crofton (1998e,f)	0, 0.1, 1.0, 3.0, 10
6-14	TSH rank order *vs.* severity rating for follicular hypertrophy; TSH rank order *vs.* severity rating for decreased follicular lumen size	F1 rat pups on PND5 (neurodevelop.) Argus Research Laboratories, Inc. (1998b); York (1998c); Channel (1998b); Crofton (1998e,f)	0, 0.1, 1.0, 3.0, 10
6-15	T3 *vs.* T4; T4 *vs.* TSH	F0 rabbits on GD29 (developmental)	0, 0.01, 1.0, 10, 30, 100
6-16	T4, TSH rank order *vs.* severity rating for follicular hypertrophy	F0 rabbits on GD29 (developmental)	0, 0.01, 1.0, 10, 30, 100

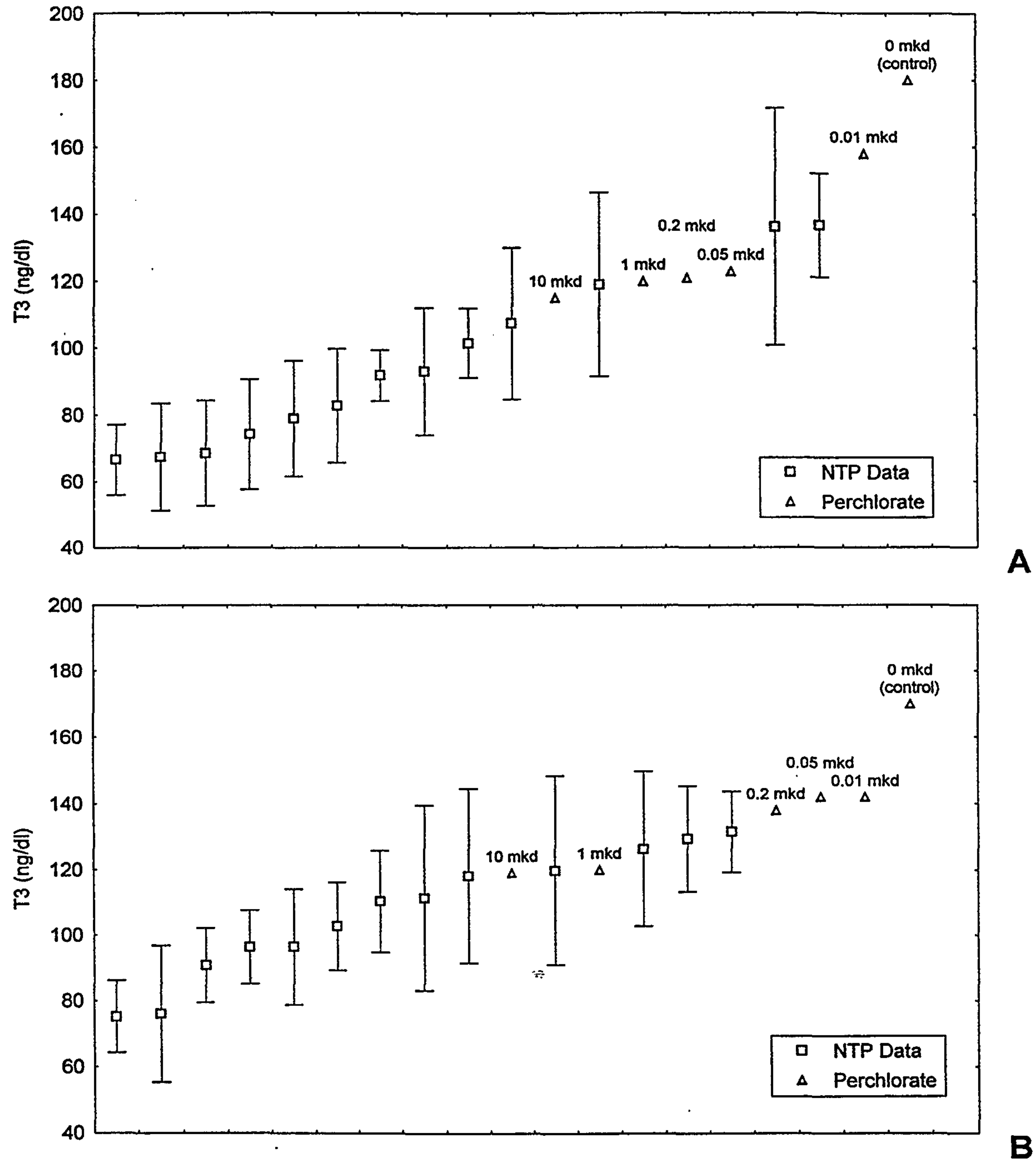


Figure 1. Serum total T3 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard deviations for the control groups in all thirteen bioassays for which reliable serum T3 measurements are available; courtesy of Dr. Greg Travlos, NIEHS. *Perchlorate data:* mean values; from Springborn Laboratories, Inc., as reported in Fig. 5-6 of the External Review Draft. *Abbreviation:* mkd, mg/kg-day.

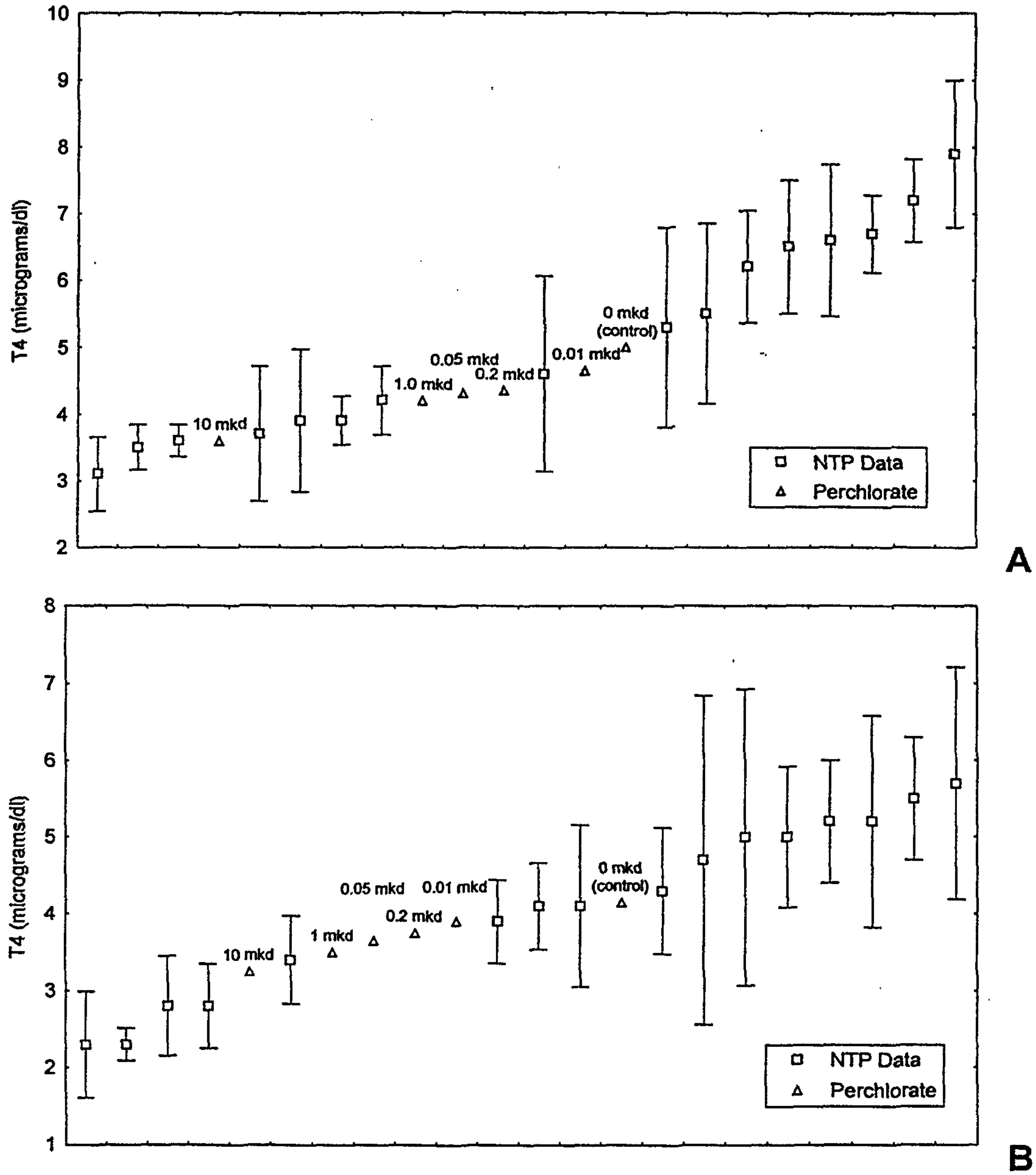


Figure 2. Serum total T4 Levels in male (**A**) and female (**B**) control rats at terminal sacrifice in 13-week NTP bioassays: Rank-order comparison with 90-day data in male (**A**) and female (**B**) rats from the 1998 subchronic exposure study of perchlorate in drinking water. *NTP data:* mean values and standard deviations for the control groups in all sixteen bioassays for which reliable serum T4 measurements are available; courtesy of Dr. Greg Travlos, NIEHS. *Perchlorate data:* mean values; from Springborn Laboratories, Inc., as reported in Fig. 5-8 of the External Review Draft. *Abbreviation:* mkd, mg/kg-day.

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1 February 1999

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RE: Comments on EPA Draft Document: Perchlorate Environmental Contamination: "Toxicological Review and Risk Characterization Based Upon Emerging Information" and Recent Mode-of-Action Studies with Ammonium Perchlorate

Dear Ms. Dardin:

The results of recent studies with ammonium perchlorate support earlier reports in the literature that the most critical effects of the compound are observed on the thyroid gland and interferes with the gland's ability to secrete thyroid hormones. This is consistent with the known mode-of-action of perchlorate as a potent inhibitor of the sodium-iodide symporter in the baso-lateral membrane of follicular cells, thereby, interfering with the ability of the thyroid gland to concentrate adequate amounts of intracellular iodide necessary for thyroid hormone synthesis to proceed at a normal rate.

I offer the following general comments for consideration by the agency as they finalize this important document.

1) It is important when interpreting thyroid hormone data and histopathologic findings of the thyroid gland, especially in the rat, to distinguish between adaptive (physiologic) responses from adverse (harmful) effects. This is particularly difficult in an endocrine organ such as the thyroid gland since it's physiologic function is to respond to changes in the internal environment of an animal or person and restore normal homeostasis. Therefore, mild (minimal) hypertrophy of follicular cells (as described in several of the recent studies) accompanied by modest elevations in circulating levels of thyroid stimulating hormone (TSH), in my judgement, should be considered an adaptive response by the thyroid gland to a mild interference in the ability to concentrate iodide ion. Similar adaptive changes are observed during pregnancy and periods of rapid growth, dietary iodine deficiency, and in response to stressful situations (e.g. low ambient temperature, severe infection), among others.

   I would suggest an adverse effect (low dose) of ammonium perchlorate to be one where there is definite evidence of follicular cell hyperplasia accompanied by a significant

Ella Dardin
1 February 1999
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increase in thyroid weights with significant reduction in both thyroxine (T4) and triiodothyronine (T3) as well as the expected compensatory increase in serum TSH. Based upon the known mode of action of perchlorate, the synthesis of both hormones (T4 and T3) should be equally interfered with when there is inadequate intracellular iodine. It is important to emphasize that all of the alterations in the thyroid follicular cells (hypertrophy or hyperplasia and increased thyroid weight) and changes in circulating levels of thyroid and pituitary hormones (decreased T4 and T3, increased TSH) following the administration of amonium perchlorate are fully reversible changes following cessation of compound administration.

2) The histologic changes described in the rat pups on post-natal day 5 should be interpreted with caution. In contrast to the pattern of change in fetal and neonatal thyroid function during pregnancy and extrauterine adaptation presented for humans in Figure 6-2, the rat thyroid may not be identical to humans. It has been my observation that the development of thyroid follicles and progressive accumulation of colloid continues into the early post-natal period in the rat making subjective histopathologic interpretation of the effects of a xenobiotic chemical on the thyroid difficult and subject to misinterpretation during this period of rapid growth. Therefore, I would not consider neonatal rats at post-natal day 5 to be an appropriate population to serve as a basis for setting the Rfd for perchlorate until this finding has been shown to be reproducible and separate from the normal developmental changes occurring at this time in the rat that result in the unique follicular structure of the thyroid gland (which is different from all other endocrine organs of the body).

It is significant to note that although mild histologic changes were observed in developing follicular cells at post-natal day 5 at the lowest dose of perchlorate (0.1 mg/kg/day), changes in serum levels of thyroid hormones and TSH were detected only at higher doses of ammonium perchlorate (T3 significantly decreased at 0.3 mg/kg/day; T4 significantly decreased at 3 mg/kg/day; and TSH significantly decreased only at 10 mg/kg/day). This lack of correlation of histologic changes in thyroid follicular cells and circulating levels of thyroid and pituitary hormones suggests to me that the described histologic change at post-natal day 5 most likely is unrelated to the administration of ammonium perchlorate.

3) Review of Table 6-1A-D and 6-2 revealed there to be considerable variability in hormone levels and thyroid histologic changes between studies and at what dose level of ammonium perchlorate an effect was detected. Some of this variability may be related to the sensitivity in measuring circulating levels of a particular hormone in a species different from that of the primary antibody of the assay (e.g. measuring mouse TSH with a rat specified TSH assay). I would suggest that another factor accounting for the lack of correlation between circulating hormone levels in thyroid structure relates to determining an adaptive from an adverse effect in response to different doses of ammonium

perchlorate (refer to item 1 mentioned previously). In addition, histopathologic evaluations of thyroid glands from studies with known thyroid active compounds, such as perchlorate, should record follicular cell hypertrophy separate from follicular cell hyperplasia (not grouped together or only recording hyperplasia as in several of the recent studies).

4) The data package on the potential thyroid toxicity of ammonium perchlorate has been greatly enhanced by the recent subchronic (90 day) toxicity study, developmental neurotoxicity study, and the two-generation reproductive toxicity study in Sprague Dawley Rats; a developmental study in rabbits; and the immunotoxicology study in B6C3F1 mice. However, studies in a non-rodent species (e.g. non-human primate or dog) would be helpful in putting the thyroid data relating to perturbations in thyroid structure and function attributed to ammonium perchlorate in perspective due to the known marked physiologic differences between the human and rat thyroid (summarized in Table 4-1). Due to the wide potential human exposure to ammonium perchlorate, data from a primate species (whose thyroid gland is known to respond to potential thyrotoxic compounds in a matter more similar to the human than the rat), in my judgement would provide important information useful in quantifying the potential risk of this compound to humans.

Thank you for the opportunity to comment on the well prepared document that the USEPA has drafted on amonium perchlorate.

Sincerely,

Charles C. Capen

Charles C. Capen, DVM, PhD
Professor and Chairman

Appendix G

List of Outside Observers Giving Presentations

PRESENTATIONS FROM OBSERVERS

Michael Dourson
Toxicology Excellence for Risk
Assessment (TERA)
Cincinnati, Ohio

Larry Ladd
Community Representative-
Aerojet Health Assessment Site Team
Rancho Cordova, California

John Gibbs
Kerr-McGee Corporation
Oklahoma City, Oklahoma

Steve Lamm
Consultants in Epidemiology and
Occupational Health, Inc.
Washington, D.C.

Kenneth Crump
K.S. Crump Group, Inc.
Ruston, Louisiana

Linda Ferguson
American Pacific Corporation
Las Vegas, Nevada

Barbara Beck
Gradient Corporation
Cambridge, Massachusetts

David Mattie
AFRL/HEST
Wright Patterson Air Force Base, Ohio

Gay Goodman
Human Health Risk Resources, Inc.
Seattle, Washington

Michael McClain
Jellinek, Schwartz and Connolly, Inc.
Arlington, Virginia

Richard Pleus
Intertox
Seattle, Washington

Dan Rogers
Wright-Patterson Air Force Base, Ohio

Deborah Keil
Medical University of South Carolina

Appendix H

Copy of Material Presented at the Meeting by Outside Observers

Larry Ladd
Aerojet Health Assessment Site Team

From: Dallas, John S. <jdallas@utmb.edu>
To: 'Larry Ladd' <llladd@sprintmail.com>
Subject: RE: Congenital Hypothyroidism and Perchlorate Water Contamination
Date: Monday, February 08, 1999 7:46 AM

Dear Mr. Ladd,

It is true that the incidence of congenital hypothyroidism is higher in Hispanics than in Afro-Americans. In Texas, Hispanics tend to have an incidence around 1/3000 and Blacks around 1/15000-16000 (about a 5-6 fold difference). While it is clear that perchlorate can interfere with thyroid function, I know of no evidence that suggests that perchlorate alone can inhibit thyroid gland formation. Do the children you discuss in your letter have thyroid glands present in the appropriate location in the neck? I might expect them to have goiters, but they should all have thyroid glands that can be detected. Has there been an increase in acquired hypothyroidism or goiter detection in this area you reference? I'm not sure what else is in rocket fuel, but I would imagine there may be several other toxins, and whether or not any of these can, in association with perchlorate, cause permanent hypothyroidism may be unknown.

I have discussed your question with Dr. Lester Van Middlesworth (Memphis, TN). He is a world's expert on environmental toxins and thyroid function in animals. He has shown in rats that in utero exposure of perchlorate can cause fetal hypothyroidism with subsequent brain damage. However, the thyroid glands developed normally in the animals; the perchlorate blocked iodine uptake by the glands and led to hypothyroidism. Once the perchlorate exposure had been removed, the thyroid function reverted to normal in these animals, but the brain damage persisted.

I hope that this information is helpful to you. Good luck.

John Dallas

> ———
> From: Larry Ladd[SMTP:llladd@sprintmail.com]
> Sent: Sunday, February 07, 1999 11:55 PM
> To: jdallas@UTMB.EDU
> Cc: Reserve Copy; Maryann Castaneda; Chris Anaya; Jane Williams
> Subject: Congenital Hypothyroidism and Perchlorate Water
> Contamination
>
> Dear Dr. Dallas,
>
> My name is Larry Ladd, and I'm a geographer serving as a community
> representative on the Aerojet Health Assessment Site Team (AHAST). AHAST
> is
> a committee of environmental regulators, Aerojet employees, and citizens
> who live next to the Aerojet Superfund Site. Our purpose is to
> investigate
> medical abnormalities that may be related to water contamination from the
> Aerojet sites in Rancho Cordova and Azusa, California. During the time
> period when Rancho Cordova's water supply became contaminated with 300 ppb

> perchlorate (1990-1996), our congenital hypothyroidism rate went up to
> 1/1300. A n of 4 cases of congenital hypothyroidism is of course not
> generalizable, but I did note that nationally there is a marked
> discrepancy
> between the Afro-American CH rate (1/30,000) and the Hispanic CH rate
> (1/2000). The reference I read suggested this difference was due to
> genetics, but in my initial search I have yet to find any evidence linking
> CH to a specific gene.
>
> I was wondering: Is there was a similar 15-fold distinction between Black
> and Hispanic CH rates in Texas? The reason I ask is that I am toying with
> the hypothesis that the high Hispanic CH rate is associated with
> perchlorate water contamination in the lower Colorado River, and the low
> Afro-American rate is partially due to the fact that soul food has no
> rocket fuel in it (e.g., preference for collared greens over Colorado
> River
> lettuce, watermelon over Colorado River cantaloupe, etc.). Any insights
> you have on this question that you can send my way would be deeply
> appreciated, especially if they come before the external peer review on
> perchlorate toxicology that will be held this Wednesday in San Bernardino,
> CA.
>
> Sincerely,
>
> Larry Ladd
>
> Community Representative
> Aerojet Health Assessment Site Team
> Rancho Cordova, California
>
> For further information on perchlorate water contamination, see
> http://www.zerowasteamerica.org/Perchlorate.htm
>

Trouble On Tap

What's In Sacramento's Drinking Water?

When Jake Parker developed a serious bone marrow infection last year, his mother, Lori, had no idea it might be linked to the water. The Parkers live next door to a Rancho Cordova well that was closed down in February due to the presence of 18 times as much perchlorate as the federal Environmental Protection Agency considers safe.

After he fell down in the driveway, Lori took 23-month-old Jake to the hospital for X-rays. Doctors didn't find anything, so they went home. But the problem did not go away.

"A week later, he was dragging his leg like a dead limb," Lori said. "He was pale. It was terrible."

Doctors finally found a bone marrow infection in Jake's shin. After many complications, and three months of antibiotics, he got better.

The Parkers say they have reason to suspect perchlorate caused their son's health problem. And they aren't the only ones. Last Friday, 100 Rancho Cordova residents filed suit against two local aerospace companies linked to the introduction of perchlorate into their groundwater.

In doses thousands of times higher than those found in Rancho Cordova's tainted wells, perchlorate can cause diseases of the thyroid, blood and bone marrow. If the substance turns out to be toxic in low doses over longer periods of time, it could be the most shocking environmental bombshell of the decade, said environmental activist Jane Williams.

Perchlorate is a chemical component of fireworks, rocket fuel and matches. The highly soluble chemical travels freely in groundwater, and no simple treatment method has been developed to remove it.

Since the closure of a number of Rancho Cordova wells last February, perchlorate has turned up all over the country. In Nevada, water flowing into Lake Mead has concentrations 100 times higher than the recommended safe levels. The lake was created by the damming of the Colorado River, which supplies drinking water to the Southwestern United States.

And perchlorate isn't the only toxin showing up in Sacramento-area water (see related stories). A plume of cancer-causing chemicals from the county dump is threatening domestic wells near Rancho Murieta. The gasoline additive MTBE has turned up in groundwater and wells wherever gas stations are located. Lead remains an ever-present threat to the safety of tap water. And the presence of nitrates has recently led to the closure of public drinking water wells in Davis.

BY AMY PARIS

PHOTOS BY LARRY DALTON

Watch That Water

Chances are, the majority of Sacramentans are drinking safe water. But as residents like Larry Ladd have found out, sometimes it pays to start asking questions.

Ladd, a geographer who lives in Rancho Cordova, stumbled onto the perchlorate situation when investigating the boundary of the Folsom-Cordova school district split. He discovered that a toxic plume, the legacy of years of chemical dumping, was creeping southwestward through the groundwater from the nearby Aeroject General Corp. plant.

Under orders from the state, Aerojet has been filtering carcinogenic chemicals such as trichloroethylene (TCE) from the *contaminated groundwater plume* since the mid-1980s, and reinjecting the water into the aquifer just upstream from Rancho Cordova. But the treatment Aerojet uses to remove other chemicals doesn't remove perchlorate. As a result, the company continues to put water that is contaminated with up to 450 times the recommended safe concentration of perchlorate back into the ground at the rate of 8 million gallons per day.

Edie Cartwright, vice president of communications for Aerojet, said the company is doing what state officials have ordered it to do. "What do you do with 8 million gallons [of treated water] per day?" she said. "There's no immediate way to clean it up."

Although perchlorate is relatively new as a health concern, the chemical itself is certainly not new to *Sacramento's groundwater*. The chemical has been detected at 1,000 times the recommended safe concentration in Aerojet wells since the 1950s, and in public wells since at least the late 1970s. Aerojet's own labs found high levels of perchlorate in one of Rancho Cordova's public wells in 1993.

"The only people who really didn't know about it were the [illegible]ple who were drinking it," [illegible] said.

State regulators overseei[illegible] Aerojet cleanup were also in the dark because, as part of its state-mandated cleanup order, Aerojet was in charge of monitoring its own wells for perchlorate. It wasn't until about a year ago that regulators realized that Aerojet's testing method was sensitive only to a level of 400 parts per billion (ppb), far in excess of EPA's recommended safe dose of no more than 18 ppb.

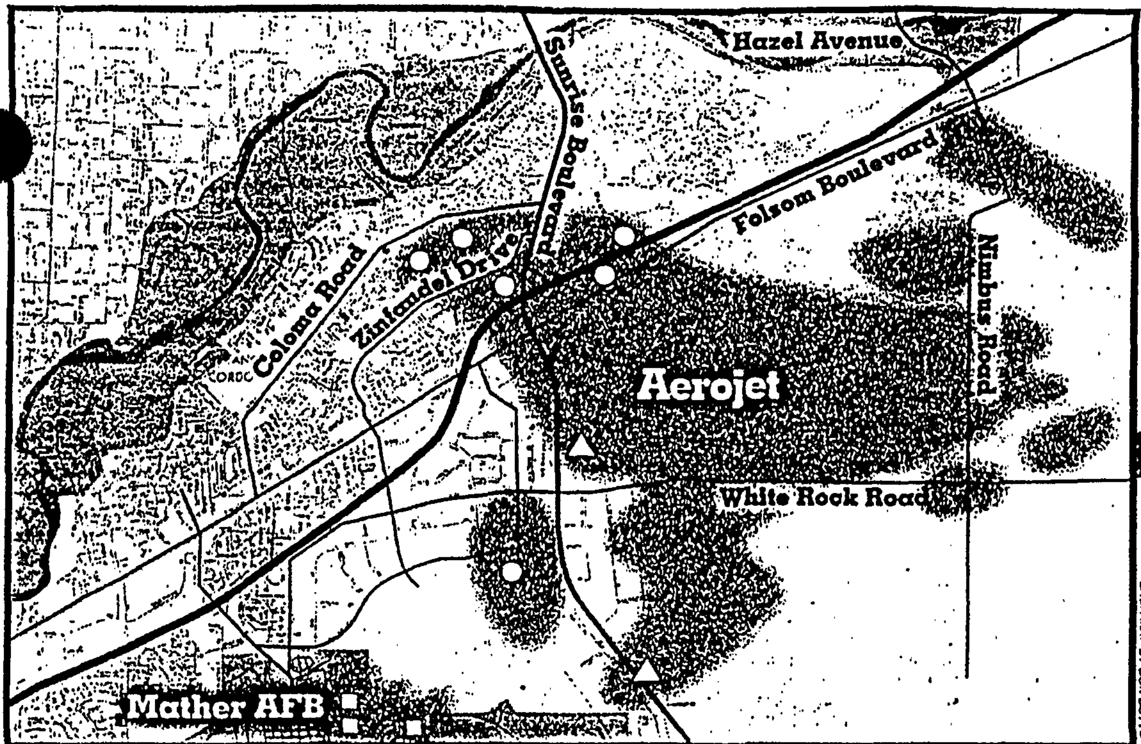
Within a matter of weeks, the state Department of Health Services laboratories developed a much more sensitive test that detected perchlorate in public wells all over Rancho Cordova.

No one knows if chronic, long-term exposure to perchlorate is hazardous because that type of exposure has not been studied.

In high doses, perchlorate can cause some pretty nasty health problems. At 1,000 times the recommended safe dosage for drinking

Lori Parker believes the tap water in her neighborhood is the cause of her son Jake's medical problems.

IS THIS THE DARK SIDE OF MEASURE O?



Shaded areas indicate places regional water officials consider to be affected by perchlorate. The contamination is moving southwestward, officials say.

○ **Arden Cordova Wells**
△ **County Sunrise Service Area Wells**
□ **Mather Wells**

No one knows if chronic, long-term exposure to perchlorate is hazardous because that type of exposure has not been studied.

water, it can cause fatal blood and bone marrow disorders. Because of its action as an endocrine disrupter, perchlorate might be linked to breast cancer or leukemia, according to Health Department toxicologist Marilyn Underwood. It can also interfere with development of the nervous system.

The chemical was used in the past as a treatment for Graves' disease, a thyroid disorder. Perchlorate inhibits the secretion of thyroid hormones, effectively slowing down the metabolism. People treated with perchlorate have reported stomach aches and skin rashes.

Almost nothing is known about the effects of perchlorate on children or developing fetuses. In general, children are more sensitive to chemical contaminants than adults.

Jake Parker isn't the only member of his family to have suffered unexplained health problems after drinking water from the tap. His 6-year-old sister has had many health problems, including anemia. And Lori Parker suffered two miscarriages in 1992. That same year, three other women on her street had miscarriages, she said.

Attorney Ed Masry filed suit Dec. 5 in Sacramento County Superior Court against Aerojet and McDonnell-Douglas, an aerospace company recently purchased by Boeing, as well as several local water utilities, on behalf of about 100 Rancho Cordova area residents complaining of health problems allegedly caused by the contaminated water. About 25 of the plaintiffs complain of thyroid conditions, while the remaining 75 have suffered various other afflictions, including cancer. The Parkers told SN&R they had not heard of the suit, but intended to contact the attorney.

Preliminary studies of hospital records that looked for unusual numbers of blood disorders, breast cancer, leukemia and babies born with severe thyroid problems among people living in areas affected by perchlorate have all turned up negative, according to Underwood. But the health department is a long way from isolating exactly who was exposed to the chemical, and even farther from determining what health effects were caused by that exposure, she said.

Perchlorate Politics

In the quest to establish a drinking water standard for perchlorate, the financial stakes are high.

The EPA decided in 1992 that it

(continued on next page)

North Sac: Got Lead?

Many Sacramentans may unknowingly be consuming hazardous levels of lead in their drinking water. In a random sampling of tap-water, city of Sacramento water authorities found that many residents were drinking hazardous levels of lead, as much as nine times what federal authorities consider safe.

Two rounds of tap water testing in 1992 detected lead in 86 and 89 percent of households in the northern part of the city of Sacramento that get their water from a public well system. No follow-up testing has been done since then.

On the whole, Sacramento water samples were within EPA guidelines. But since lead contamination depends in some measure on the condition and chemical composition of each household's plumbing, residents should not assume their water is safe.

Lead is the leading environmental hazard for children, according to a recently released report from the National Resources Defense Council. Drinking water makes up about 20 percent of an average adult's exposure to lead and up to 85 percent for some bottle-fed infants.

People who live in houses built between 1982 and 1986 are at an increased risk for lead exposure because during those years, most copper pipes were soldered with lead. Brass faucets, which can legally contain up to 8 percent lead by weight, are leading contributors of lead to tap water.

Adding fluoride to water doubles its lead content by making the water more corrosive, according to EPA toxicologist Bill Marcus. Only a small number of Sacramentans in the Rosemont area have fluoridated water, but recent legislation has made countywide fluoridation an imminent possibility.

Lead makes you stupid, and that might be the nicest thing that it does. In addition to impairing mental and physical development, it can increase blood pressure and damage hearing, and at very high levels can cause anemia, kidney damage and mental retardation. Consumption of lead by pregnant women directly exposes the developing fetus, and can result in low birth weight and premature birth.

Running water for up to three minutes or until it becomes cold, especially the first time you use it in the morning, reduces lead levels by clearing stale water out from the pipes. Also, using cold water for cooking reduces lead exposure, since lead is more soluble in hot water. Boiling water does not remove lead.

The only way to find out for sure if lead is in your tap water is to have it tested by a state or EPA certified lab. Tests cost from $15 to $35, and the EPA Drinking Water Hotline (1-800-426-4791) can help you locate a certified lab in your area.

—A.P.

(continued from previous page)

did not have enough information about perchlorate to establish an enforceable drinking water standard, so instead it established a recommended safe dose of 4 parts per billion (ppb). By this time, Aerojet had already found almost 10,000 times this concentration in its own monitoring wells.

Aerojet and a group of businesses that manufacture and use perchlorate, fearing responsibility for an enormous and costly cleanup, formed the benignly named Perchlorate Study Group to pressure the EPA to relax its reference dose.

In 1995, the group got the EPA to expand the standard to 4-18 ppb, but considering they were shooting for much higher levels (at least 400 ppb), it was not the coup they had hoped for.

The EPA refused to reconsider the reference dose without conducting more studies. The Perchlorate Study Group and the Air Force—two groups that stand to bear the financial responsibility of cleanup—volunteered to fund studies to fill in the information gaps. The studies, which are already under way, could have a substantial effect on the determination of an EPA drinking water standard.

"The Air Force is funding a series of toxicity tests to convince the world that perchlorate is good for you," said Jane Williams, director of the California Communities Against Toxics. "There's an incestuous relationship there: They've been funding their own tests, hiring their own toxicologists, putting their own scientists on peer review panels. There is no public advocacy group watching the level changes. It's being done in the dark."

Taking scientific research as a hostage in the battle over cleanup costs is not unique to the perchlorate situation.

Larry Ladd and his daughter, Melody.

For example, last April, when federal government researchers invited Sacramento to participate in one of the most comprehensive groundwater studies ever undertaken, the city initially refused to participate. The reason: Officials wanted a guarantee they wouldn't be held liable for cleanup costs, should new contamination be found. City government changed its mind only when local media coverage incited a moderate amount of public outrage.

Since state regulators became aware of perchlorate in Sacramento area wells last February, a total of eight wells have been taken out of service, and three more were initially closed and have since reopened.

Hardest hit was Arden-Cordova Water Service, which shut down three wells showing levels from 140 to 320 ppb. Three more wells that are within the safe dosage range were initially taken offline and returned to service when the summer peak water demand came. Another well with levels within the safe range was put on standby in November.

Two wells in the county of Sacramento's Sunrise Boulevard service area are contaminated with 92 and 280 ppb, respectively. The wells are on standby, which means they kick in only in periods of high demand. One well pumped nearly 2 million gallons in May alone. The other pumped more than 120,000 gallons in August. Customers were notified of possible exposure.

In addition, two wells on Mather Air Force Base have been closed down, and a third is near the safety limit.

Anyone using private wells in the area of the plume (see map, previous page) should contact local health authorities, said Dave Lancaster of the Department of Health Services.

Water from the city of Sacramento, Fair Oaks and Folsom South Canal has not been contaminated.

A neighborhood served by Citizens Utilities is directly in the path of the plume, and the company has turned off one well because it detected trace amounts of perchlorate. The company has already been hit by a trichloroethylene-containing plume from Mather Air Force Base.

"The plume is moving our way, and we're watching it closely," said Robert Roscoe, managing engineer for Citizens Utilities. "It's like being tied to a railroad track and seeing a single light off in the distance. We'd not be doing a good jo[illegible]ing our water supply if w[illegible] plan-ning for it."

Who Pays?

So far, Aerojet has paid an estimated $3 million-$5 million for numerous interconnections between water systems and additional storage reservoirs to reduce the level of perchlorate in local water.

Some officials say Aerojet considers it the Air Force's responsibility to pay the company back, at least in part, since the Air Force played a large role in perchlorate contamination. Edie Cartwright, vice president of communications for Aerojet, would only say that the company is in negotiations with the Air Force.

In addition, if the EPA's recommended dose is raised, local water purveyors might have to compensate Aerojet for work done on their systems.

"They picked up the cost for these projects, and if the safe level is actually above what's in the wells, they're probably going to negotiate for reimbursement," said John Coppola of the Sacramento County Water Resources Division.

But the real costs are not going to come from piecemeal system improvements. A treatment plant that will extract and treat water at the contamination site is being developed at an estimated cost of $210 million. It is scheduled to begin operation next April.

Until that time, under state orders, Aerojet will continue to reinject water containing perchlorate into the aquifer. Although the planned treatment is intended to stop the reintroduction of contaminated water, it will do nothing for the fringes of the plume, which have already spread over Rancho Cordova and are heading southwest. If EPA establishes a drinking water standard that is as low as the current recommended dose, water purveyors will have to find a way to treat individual wells. That technology is years away, Regional Water Quality Control Board officials say. Meanwhile the spread of perchlorate could threaten other wells and further tax the already burdened drinking water supply.

The Parkers, who have lived in Rancho Cordova for 11 years, aren't sticking around to see how it all turns out.

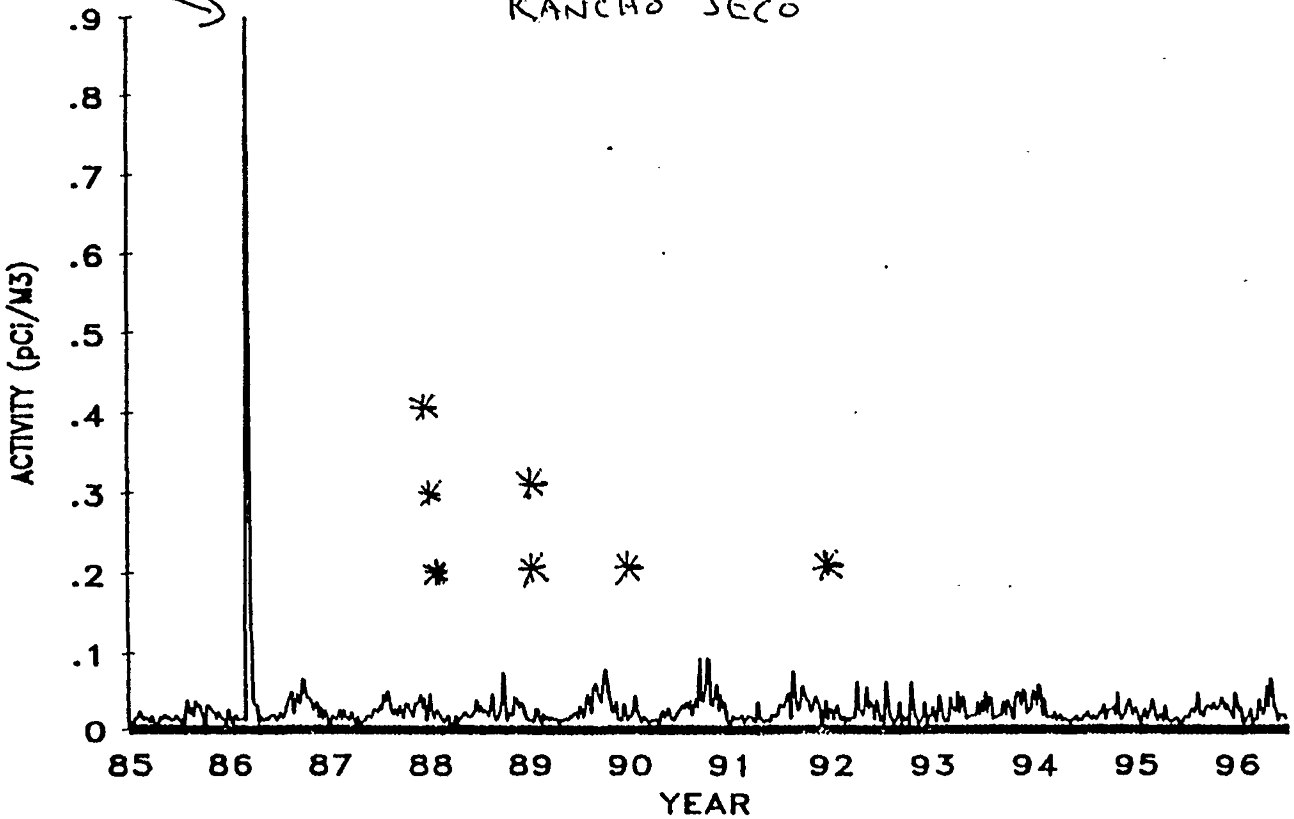
"We're putting our house on the market," Lori said. "We don't want to live here any more. It's scary being near Aerojet. Buffalo Creek—who knows what they might have washed through there? There have been a lot of unexplained health problems." □

CHERNOBYL : PERCHLORATE RESONANCE ?:

THYROID CANCER CASES IN RANCHO CORDOVA CENSUS TRACT WITH DRINKING WATER CONTAMINATED WITH PERCHLORATE AND NITROSODIMETHYLAMINE. EXPECTED FREQUENCY OF THYROID CANCER IN THE TRACT IS ONCE EVERY SEVEN YEARS.

FIGURE 5
GROSS BETA IN AIR PARTICULATE
MEAN WEEKLY ACTIVITY
ALL STATIONS
RANCHO SECO

IODINE 131
FALLOUT FROM CHERNOBYL



✳ = one thyroid cancer case

John Gibbs
Kerr-McGee Corporation

When Available, Human Health Data Should Be Used To Assess Human Health Risks

KERR-McGEE CORPORATION

Perchlorate Production & Natural Occurence

KERR-McGEE CORPORATION

- Both production facilities located in the desert
- Low humididy (<50%) necessary for handling dry product

KERR-McGEE CORPORATION

- AP has high hygroscopicity
- Packaged to exclude any moisture

KERR-McGEE CORPORATION

Workers at both of these production facilities have recently been studied and reported

- *Gibbs et al* - 1998 (Kerr-McGee - Nevada)
- *Lamm et al* - 1999 (WECCO - Utah)

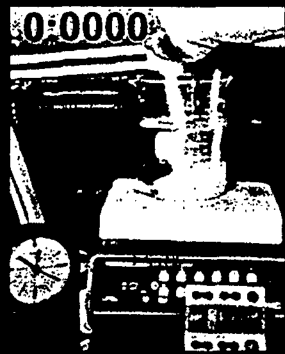
KERR-McGEE CORPORATION

Perchlorate Exposure in the Workplace

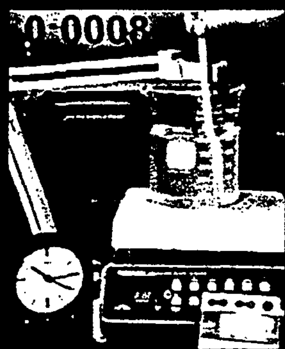
- Primarily through the inhalation of dust
- All exposures well below OSHA PEL's

KERR-McGEE CORPORATION

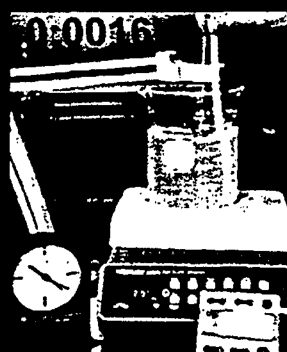
EPA questioned the validity of solubility assumptions in Gibbs et al



20 grams of ammonium perchlorate (large crystals)



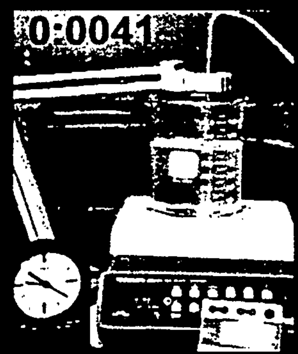
Mixed in 500 ml water



at 37°C

KERR-McGEE CORPORATION

Completely dissolved in less than one minute

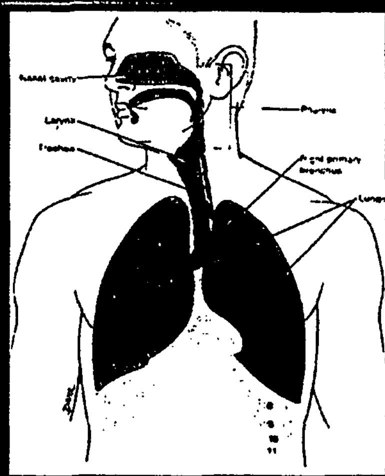


KERR-McGEE CORPORATION

- Ammonium perchlorate is rapidly soluble in water
- Solubility is about the same as table salt at body temperature

KERR-McGEE CORPORATION

EPA expressed concerns about particle size



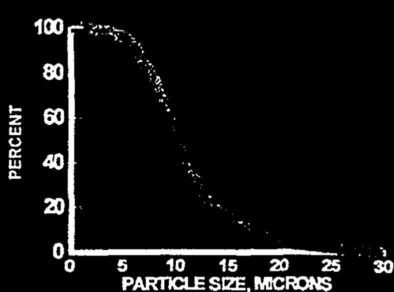
KERR-McGEE CORPORATION

- Finished product is >100 micron particles
- Dust in the workplace is much smaller
- Most dust generated in the screening and cross-blending processes
- Fines removed from product (5% sent for recrystallization)

KERR-McGEE CORPORATION

Exposure Assessment

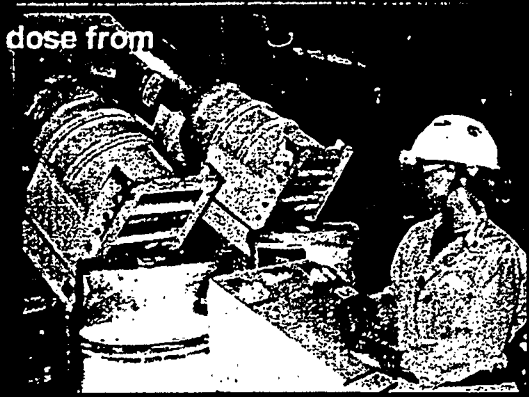
- NIOSH method 0500
- Closely approximates thoracic dust



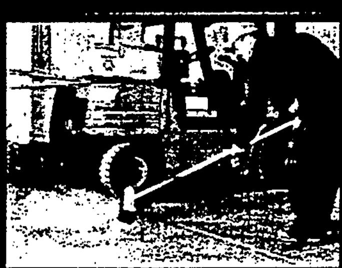
KERR-McGEE CORPORATION

Gibbs et al

- Assessed exposures to thoracic dust
- Estimated dose from exposure



KERR-McGEE CORPORATION



Lamm et al

- Exposure assessment - both thoracic and respirable dust
- Particle size characterization (MMAD 4-12 microns)

KERR-McGEE CORPORATION

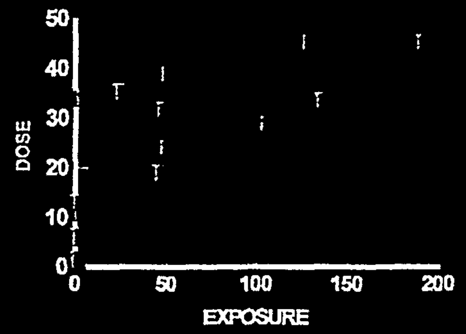
Lamm et al

- Urinary perchlorate excretion rates
- Absorbed doses calculated

KERR-McGEE CORPORATION

Lamm et al

- Strong exposure / dose relationship
- Validates exposure assumptions in Gibbs et al



Lamm et al & Gibbs et al

86 workers averaging 7 years exposure

- No thyroid effects
- No hematological effects
- No renal or hepatic effects

BMDL 0.6 - 0.8 mg/kg-day based on TSH & FTI

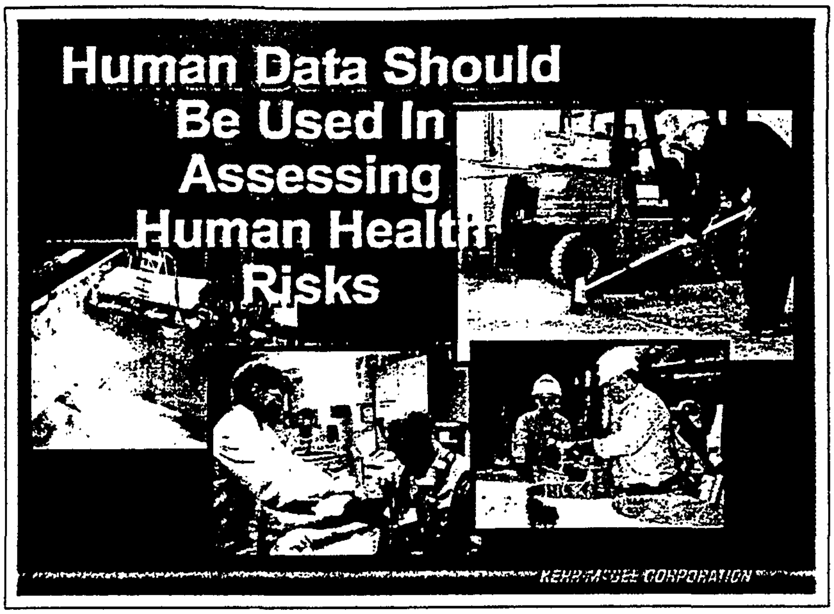
EPA Did Not Use These Studies In Developing RfD

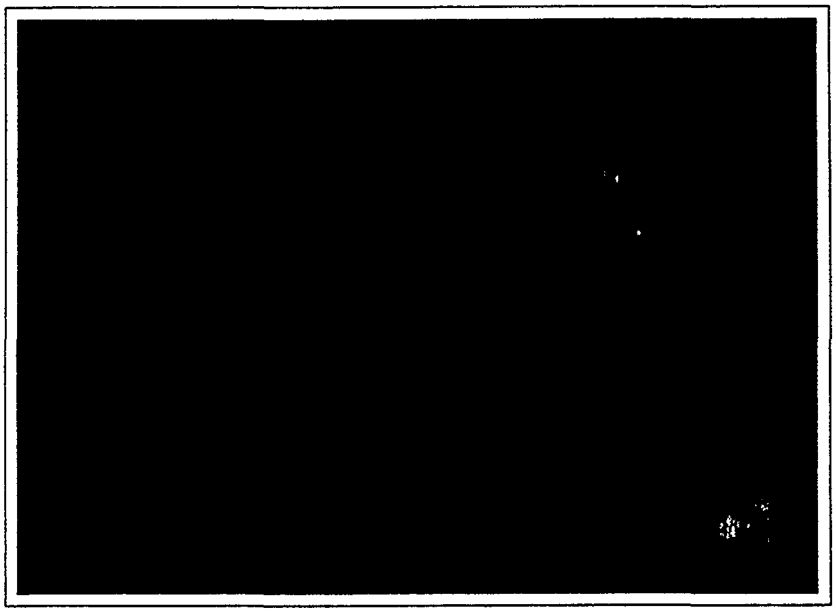
Lam. et al

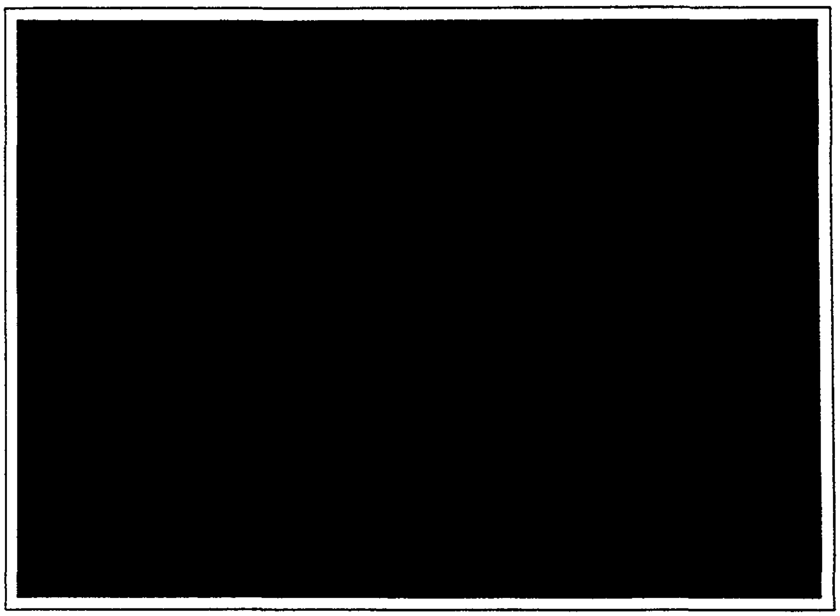
- Not yet accepted for journal publication
  - Accepted by JOE January 1999

Gibbs et al

- Concerns about dose estimates based on exposure
  - EPA should reconsider particle size, exposure measurements and solubility
  - Lam. et al confirmed exposure/dose relationship


Human Data Should
Be Used In
Assessing
Human Health
Risks
KERR-McGEE CORPORATION





Steve Lamm
Consultants in Epidemiology and Occupational Health, Inc.

Human Health Effects of Perchlorate

Reports prepared by Consultants in Epidemiology and Occupational Health, Inc.

Medical Literature Review
Occupational Study
Congenital Hypothyroidism Study

Sponsored by American Pacific Corporation

February 10, 1999
San Bernadino, California

Perchlorate Health Effects

- After nearly 50 years of clinical experience with perchlorate we have:
  - Clear understanding of effect level and limits of toxicity
    - Treatment levels at 200 (+) mg/day
    - Hematotoxicity at > 450 mg/day, usually 1,000 (+) mg/day
  - Its mechanism of action is well understood
    - Blockage of iodine uptake at the basolateral membrane, specifically, the Na-I symporter

Perchlorate Health Effects

- No change in thyroid hormone or TSH levels seen up to exposures of 70mg/day absorbed
- Grouped data yields NOAEL at 34mg/day
- Individual data yields BMDL of 44-58 mg/day (about 51mg/day)

Perchlorate Occupational Health Study (Lamm et al., 1999)

Study Population:

58 employees at WECCO, Cedar City, UT (July 15-17, 1998)

37 Perchlorate employees (35 males; 2 females; mean age 30; 40% employed > five years)

High	15 employees
Medium	8 employees
Low	14 employees

21 Azide employees (19 males; 2 females; mean age 35; 50% employed > 5 years)

Study Design:

Each worker studied across one of six twelve-hour shifts covering three consecutive days.

Added 2/22/99

Perchlorate Occupational Health Study Measures

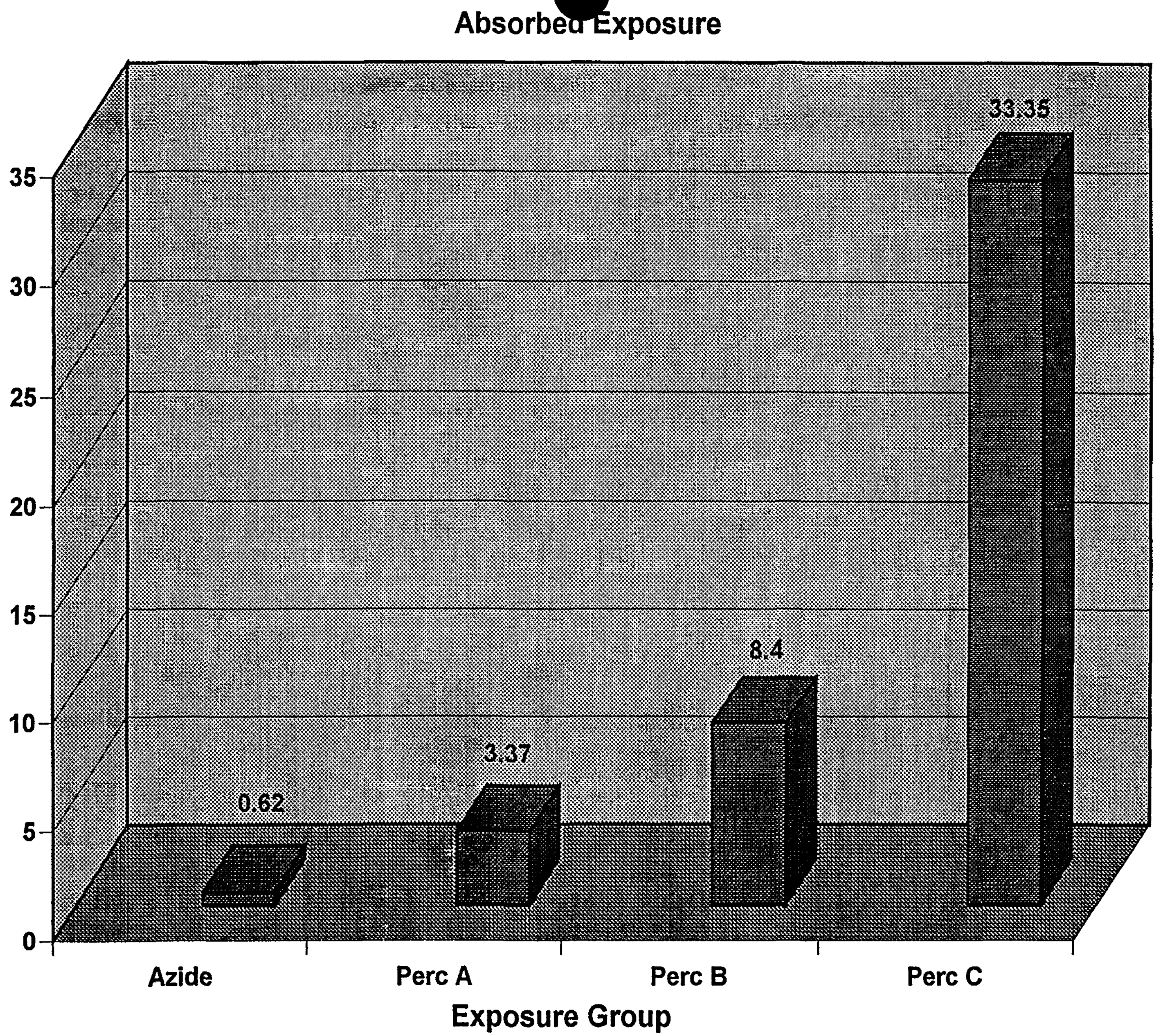
Exposure Measures:

1. Pre-shift urine perchlorate (n=58) [mg/gm creatinine]
2. Cross-shift breathing zone respiratory (n= 38) and/or total (n=24) perchlorate particulate exposure [mg/day]
3. Particle size distribution [MMAD = 7.4 um]
4. Post-shift urine perchlorate (n=58) [mg/gm creatinine]
5. Absorbed perchlorate [mg/day]

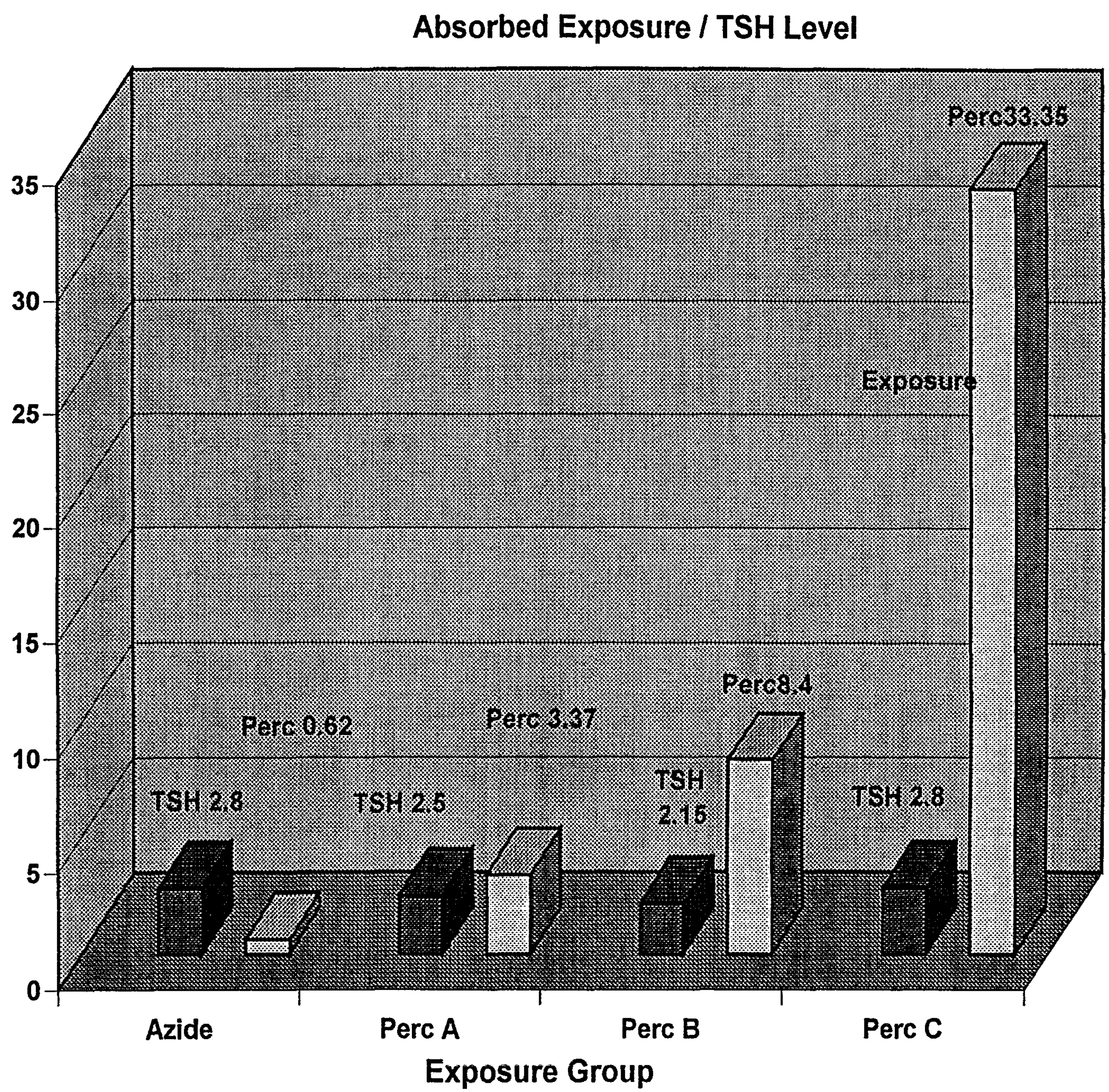
Outcome Measures:

1. Post-shift thyroid function tests (n=57)
   TSH, T_4, T_3, FTI, THBR, and Anti-TPO
2. Post-shift complete blood cell count (n=57)
   RBC, WBC, Platelets, Neutrophils, Lymphocytes, etc.
3. Post-shift blood chemistries (n=57)
   Liver function tests
   Renal function tests
   Metabolic status tests
4. Clinical thyroid examination (n=58)

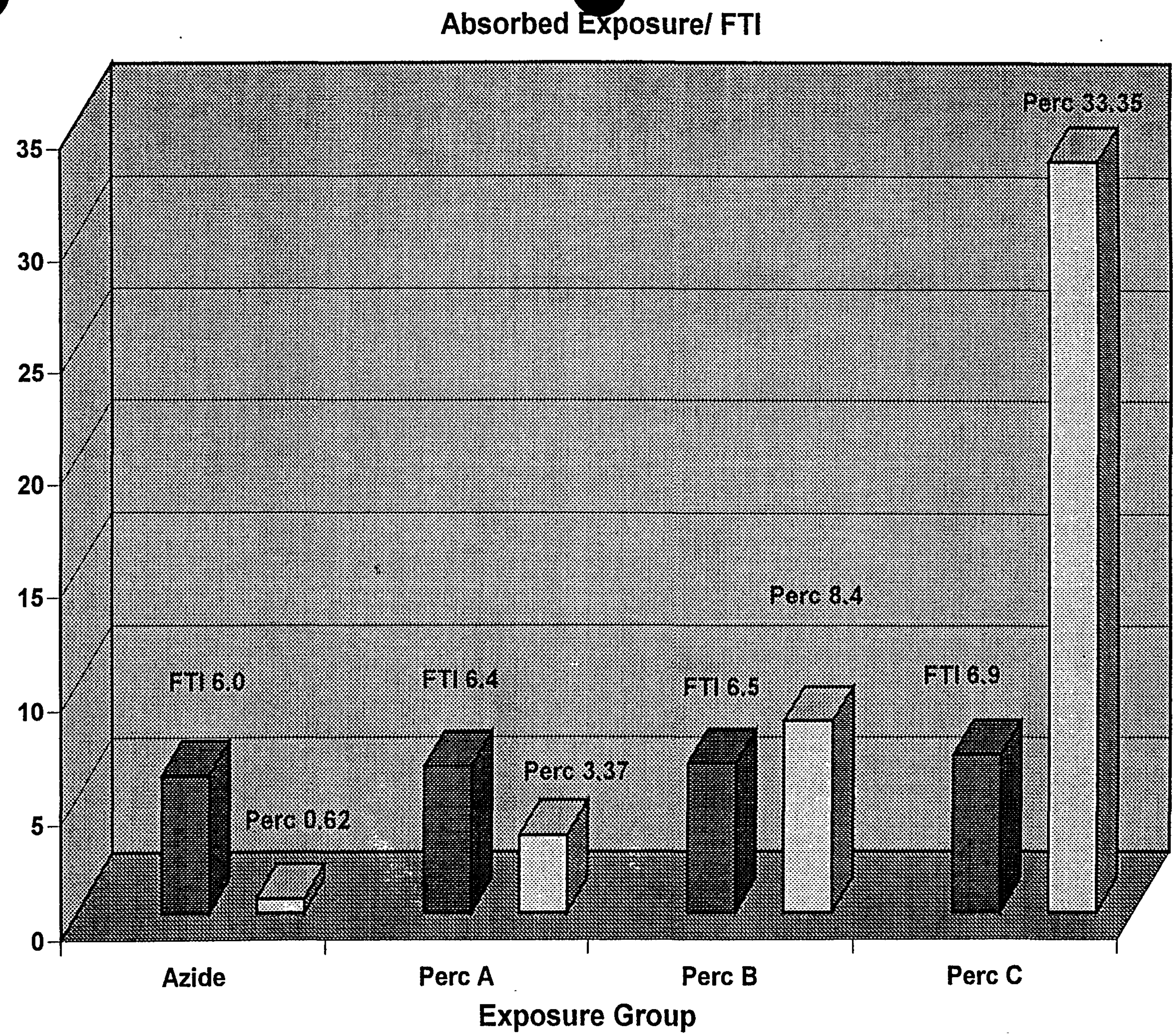
Added 2/2



Absorbed Exposure
35
30
25
20
15
10
5
0
0.62
3.37
8.4
33.35
Azide
Perc A
Perc B
Perc C
Exposure Group



Absorbed Exposure / TSH Level
35
30
25
20
15
10
5
0
Perc33.35
Exposure
Perc8.4
Perc 0.62
Perc 3.37
TSH 2.8
TSH 2.5
TSH
2.15
TSH 2.8
Azide
Perc A
Perc B
Perc C
Exposure Group

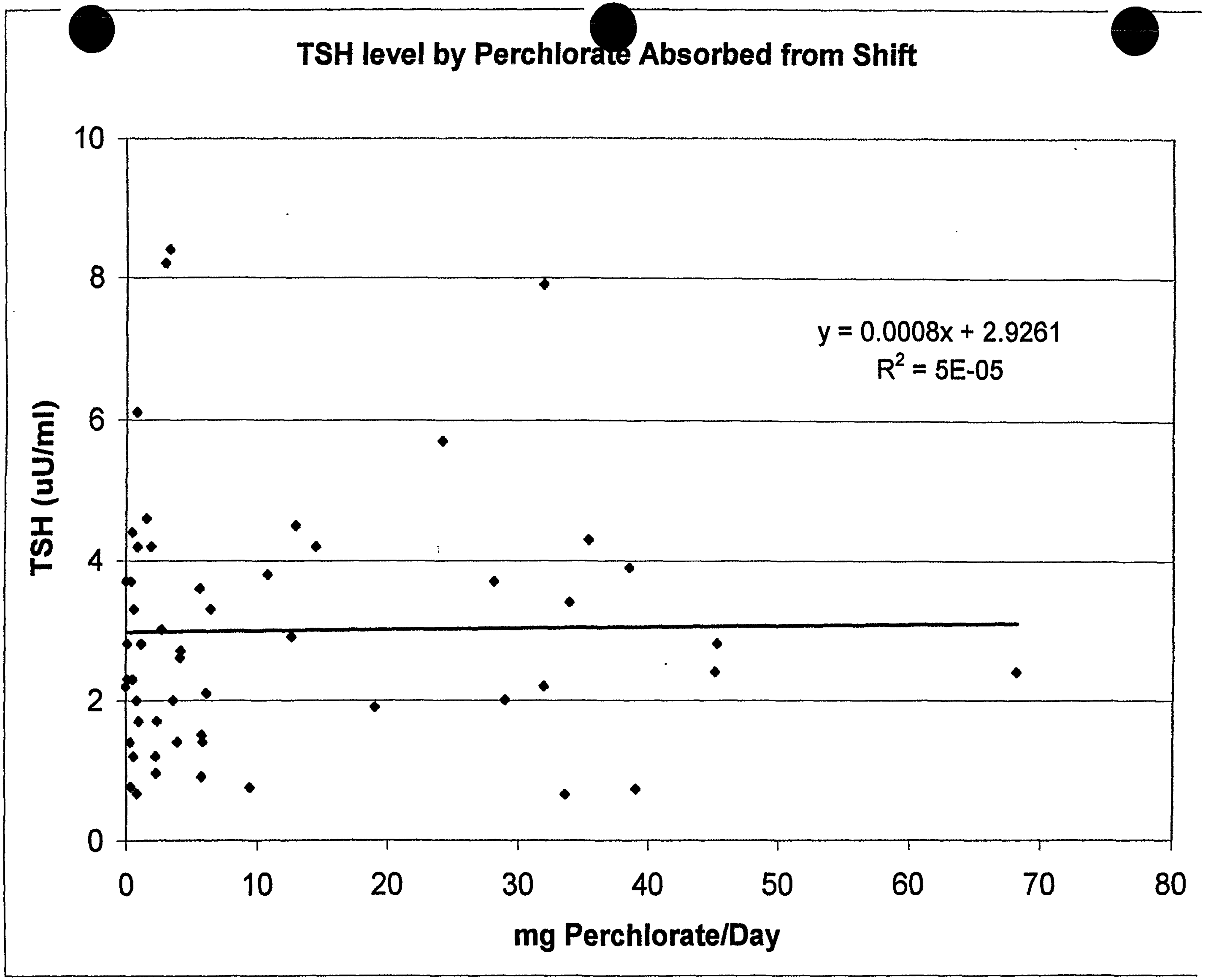

Absorbed Exposure/ FTI
Perc 33.35
Perc 8.4
FTI 6.0
FTI 6.4
FTI 6.5
FTI 6.9
Perc 3.37
Perc 0.62
35
30
25
20
15
10
5
0
Azide
Perc A
Perc B
Perc C
Exposure Group

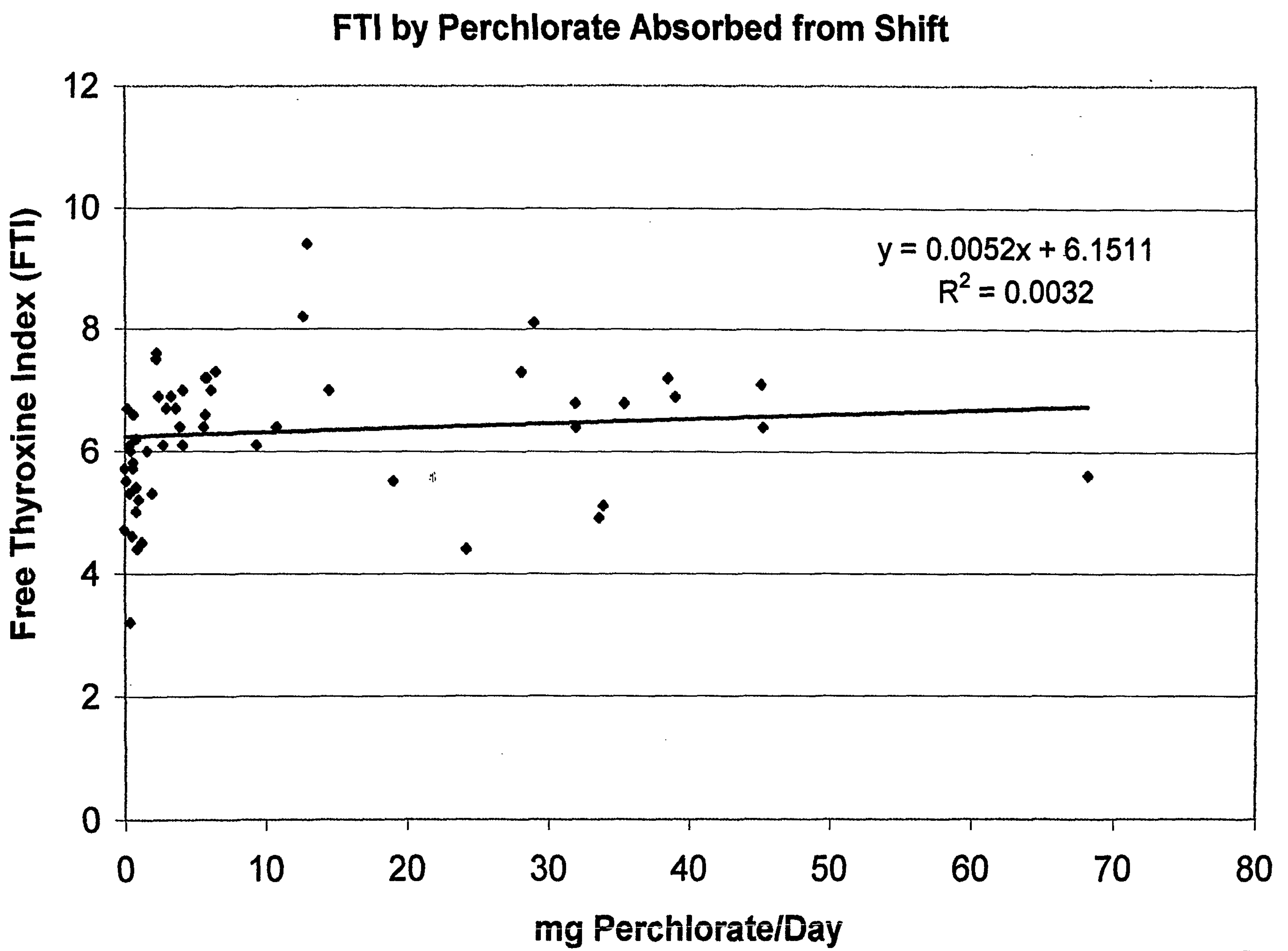
8

Perchlorate Absorption

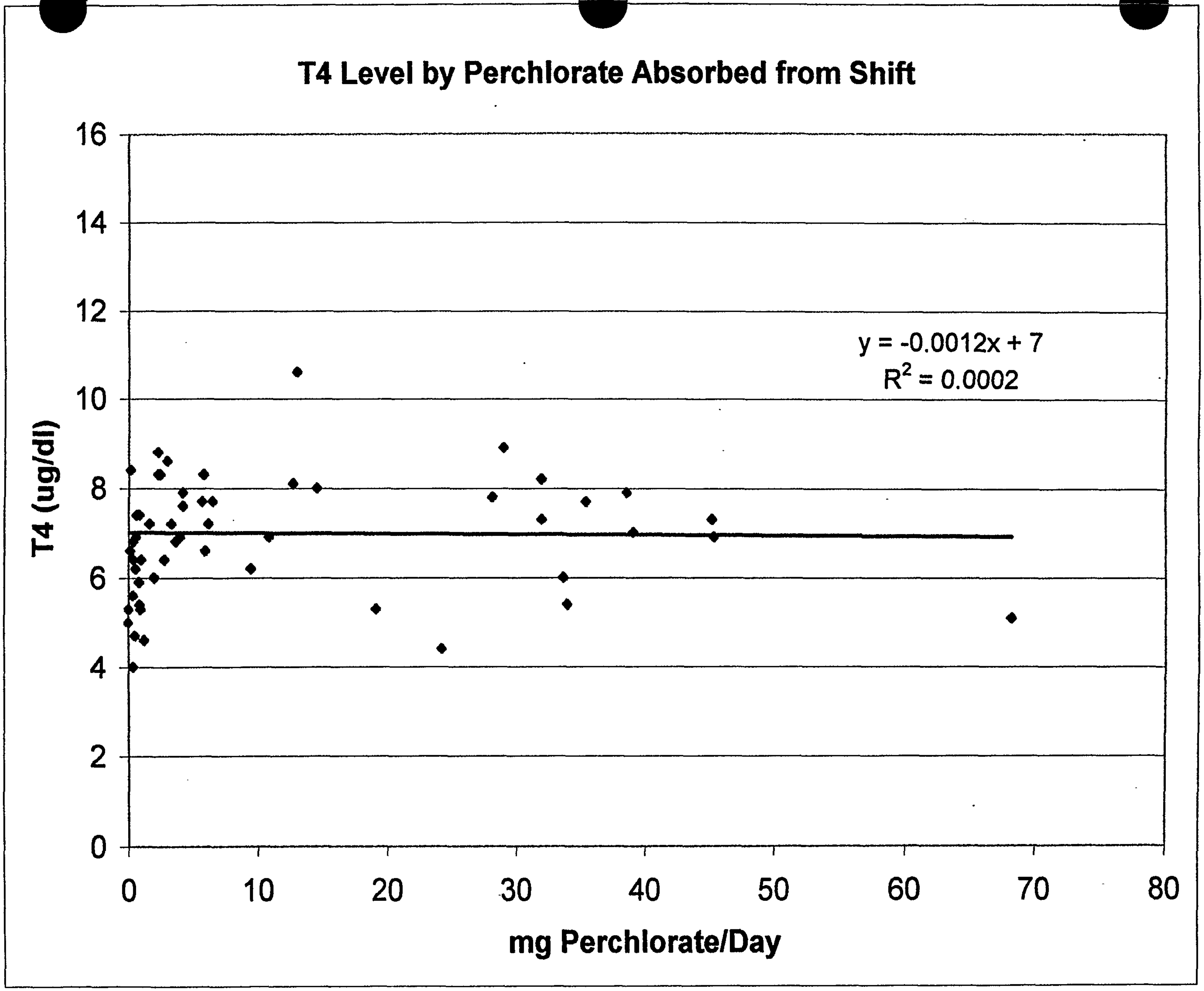
Expected Effects

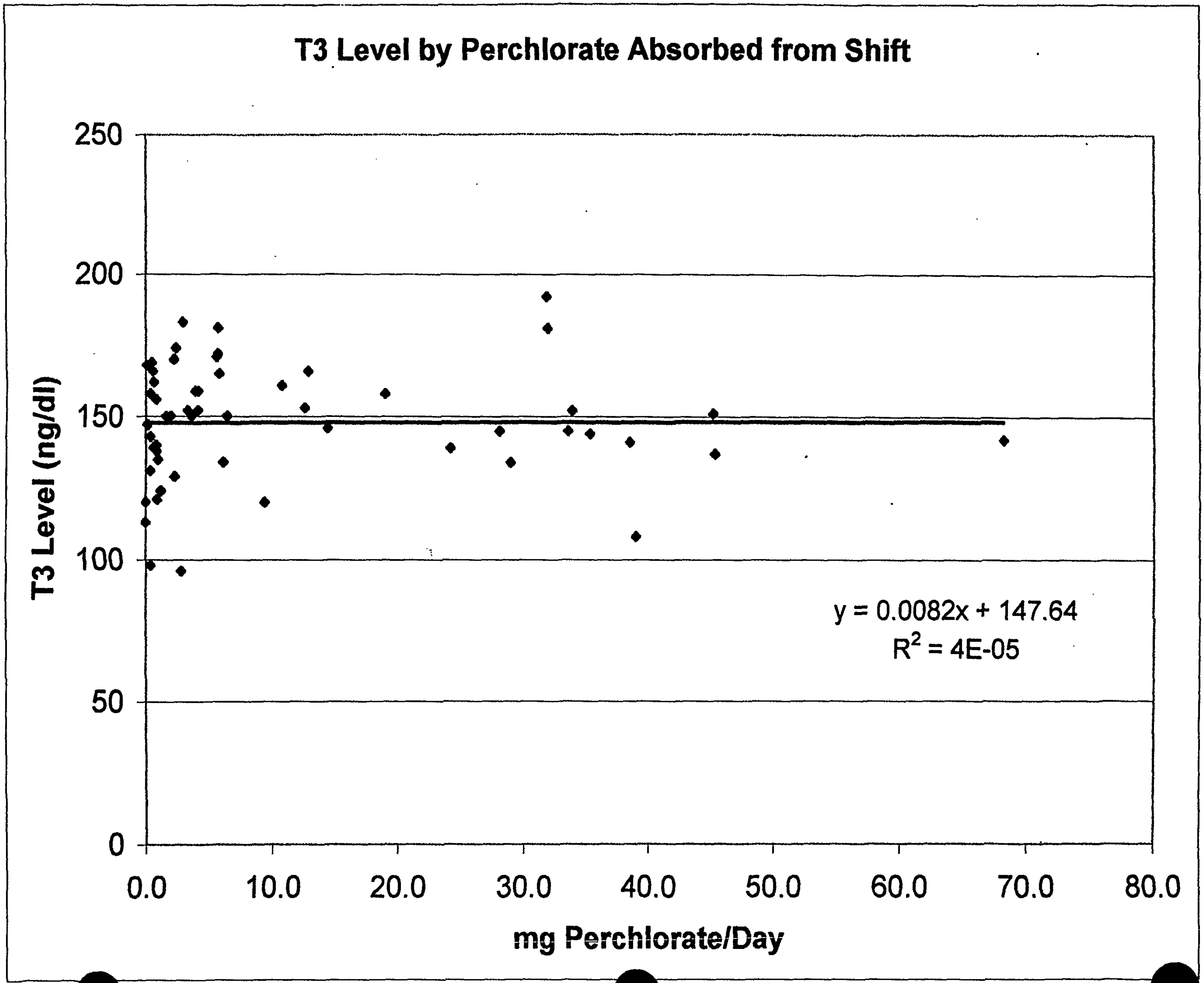
TSH	↑
FTI	↓
T_4	↓
T_3	↓
THBR	↓


TSH level by Perchlorate Absorbed from Shift
y = 0.0008x + 2.9261
R2 = 5E-05
TSH (uU/ml)
mg Perchlorate/Day
0
2
4
6
8
10
0
10
20
30
40
50
60
70
80

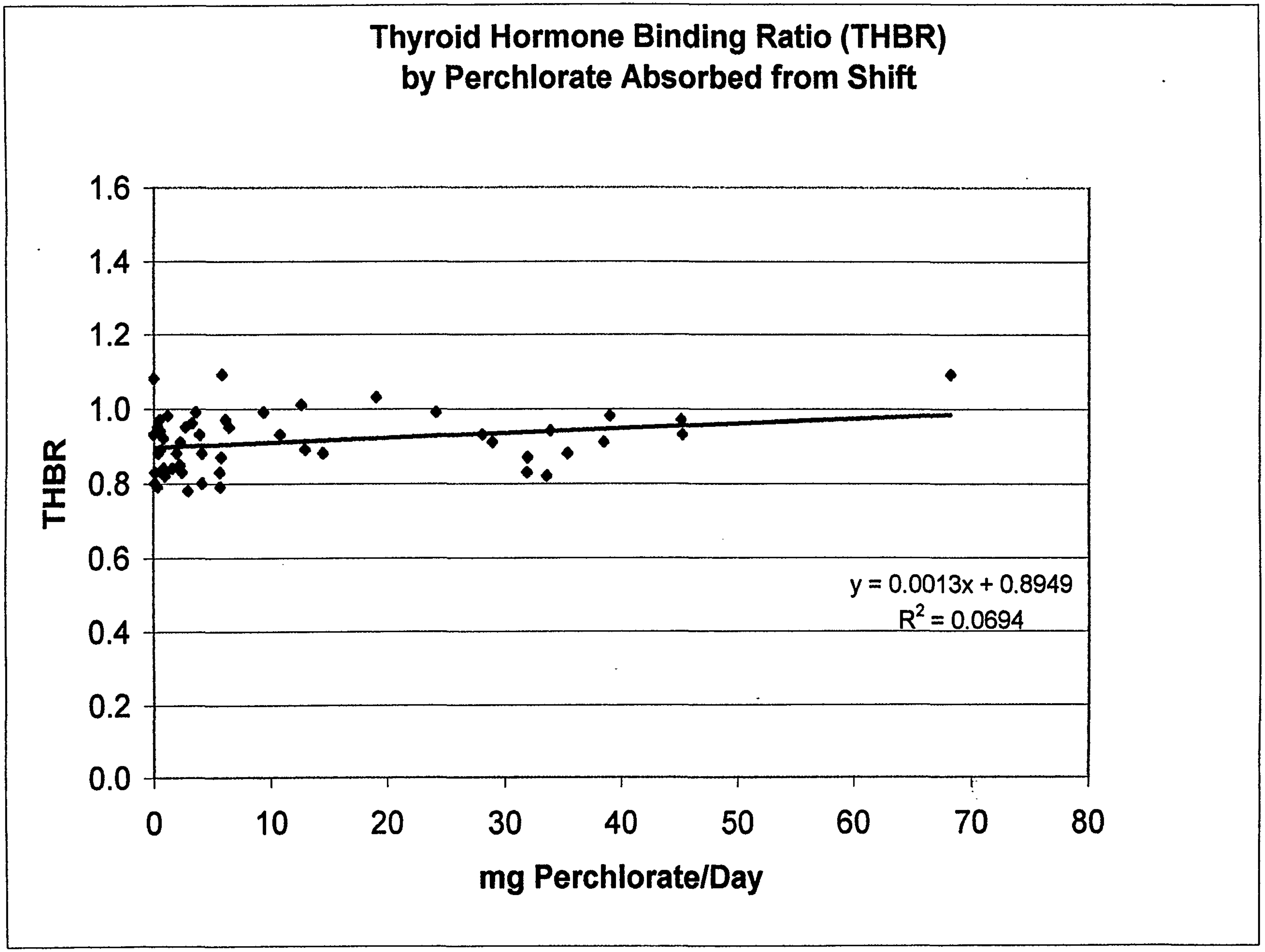


FTI by Perchlorate Absorbed from Shift
y = 0.0052x + 6.1511
R2 = 0.0032
Free Thyroxine Index (FTI)
12
10
8
6
4
2
0
0
10
20
30
40
50
60
70
80
mg Perchlorate/Day


T4 Level by Perchlorate Absorbed from Shift
y = -0.0012x + 7
R2 = 0.0002
T4 (ug/dl)
mg Perchlorate/Day
0
2
4
6
8
10
12
14
16
0
10
20
30
40
50
60
70
80


T3 Level by Perchlorate Absorbed from Shift
250
200
150
100
50
0
T3 Level (ng/dl)
y = 0.0082x + 147.64
R2 = 4E-05
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
mg Perchlorate/Day

13


Thyroid Hormone Binding Ratio (THBR)
by Perchlorate Absorbed from Shift
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
THBR
y = 0.0013x + 0.8949
R2 = 0.0694
0
10
20
30
40
50
60
70
80
mg Perchlorate/Day

14

Perchlorate Absorption

Observed Effects
(0-70 mg/day)

TSH ➔

FTI ➔

T_4 ➔

T_3 ➔

THBR ➔

Perchlorate Absorption

Expected Effects		Observed Effects	
TSH	↑	TSH	→
FTI	↓	FTI	→
T_4	↓	T_4	→
T_3	↓	T_3	→
THBR	↓	THBR	→

Perchlorate Health Effects

- No evidence of increase in congenital hypothyroidism at recent environmental exposure levels.

California and Nevada
Seven Counties
(1996 – 1997)

Screened Newborns	691,208
Observed	249 cases
Expected	243 cases
Ratio	1.03 (0.90-1.16)
p-value	0.63

Congenital Hypothyroidism Cases (Observed and Expected*) for 1996 and 1997 in Nevada and California Counties with Perchlorate Reported in Water Supply

			Congenital Hypothyroidism Cases			
State	County	Newborns Screened	Observed	Expected	Observed/ Expected	95% Conf. Limits
Nevada	Clark	36,016	7	8.3	0.84	(0.34-1.74)
California	Los Angeles	338,934	136	123.5	1.10	(0.92-1.30)
	Orange	101,227	40	35.9	1.12	(0.80-1.52)
	Riverside	43,577	11	15.6	0.71	(0.35-1.26)
	Sacramento	39,235	8	12.9	0.62	(0.27-1.22)
	San Bernardino	51,637	17	18.4	0.92	(0.54-1.48)
	San Diego	80,582	30	28.2	1.06	(0.72-1.52)
	Total	655,192	242	234.6	1.03	(0.90-1.16)
Nevada and California		691,208	249	242.9	1.02	(0.90-1.16)

* Expected numbers have been adjusted for Hispanic ethnicity.

2/9/99

Linda Ferguson
American Pacific Corporation

Comments to be presented to the External Peer Review Panel, Perchlorate Technical Workshop, San Bernadino, CA, February 10, 1999.

My name is Linda Ferguson, Vice President of American Pacific Corporation. Our headquarters are located in Southern Nevada. For approximately 30 years, and until 1988, we operated a manufacturing facility that produced ammonium perchlorate, not far from the present BMI complex of industrial facilities. At the current time, our principal operating facilities and offices are located in Southern Utah. We are the sole supplier of ammonium perchlorate in the U.S.

Before I continue, let me offer you our appreciation for agreeing to serve on such an important external review panel. I think we all agree that by making this process public and accessible to all, we have the best opportunity for a result that is reasonable and supportable. We especially appreciate the fact that you have taken time from your professional careers to participate.

We are a company of approximately 220 employees, 190 of whom live and work in Utah. Many of our Nevada employees live in Henderson and join us in our concern for the citizens of Clark County regarding the quality of our drinking water.

The Company has expended significant money in attempting to contribute to a full scientific review of perchlorates, so that the true significance can be understood, and public policy formulated, based on sound scientific knowledge. The company has tried to be very responsive to government agencies and we have therefore made extensive hydrogeologic evaluations of our former plant site in Henderson.

In the summer of 1998, American Pacific conducted and reported to EPA a human health study of our own production workforce. We found no evidence of thyroid problems, as reported by CEOH. Incidentally, our employees volunteered for the study and they are interested in the results of your work.

We believe it to be imperative that you give at least equal weight and consideration to the human health studies, some of which are still underway. The studies have been funded at great expense to our company and others. We believe these studies are essential in providing adequate information to protect our community and our employees.

American Pacific, both as a corporate citizen and as individual employees, resides in Clark County, Nevada. We have serious concerns that the public must be protected from any hazards; however, we have equally large concerns that the public not be subjected to undue alarm from premature establishment of a value that was established without incorporation of available science. It is essential that the public be informed. However, publication of a reference dose that does not consider adequately all of the applicable and available science would create a number that may be subject to many revisions. Revisions cause public consternation and lower public trust both in governmental agencies and in corporations. It is essential that stakeholders in this process recognize the impact of their actions in establishing a reference dose.

The responsibilities faced by you as a peer review panel are critical. We wish to congratulate you on the contribution you are making as citizens by serving on the panel. We trust that you will be sensitive to the issues we and others have raised and award them due consideration. Thank you for your attention.

Referenced Studies

Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational Health Study

> *Has Perchlorate in Drinking Water Increased the Rate of Congenital Hypothroidism?*
>
> *Recommendations to the US EPA Concerning the Derivation of a Reference Dose for Perchlorate*

Evaluation of a Population with Occupational Exposure to Airborne Ammonium Perchlorate for Possible Acute or Chronic Effects on Thyroid Function - Gibbs, et al

The Effect of Low Dose Perchlorate on Thyroid Function - Braverman, PSG

Pharmacokinetic Study of Perchlorate Administered Orally to Humans - Brabant

Barbara Beck
Gradient Corporation

Comments on EPA Perchlorate RfD Draft Document

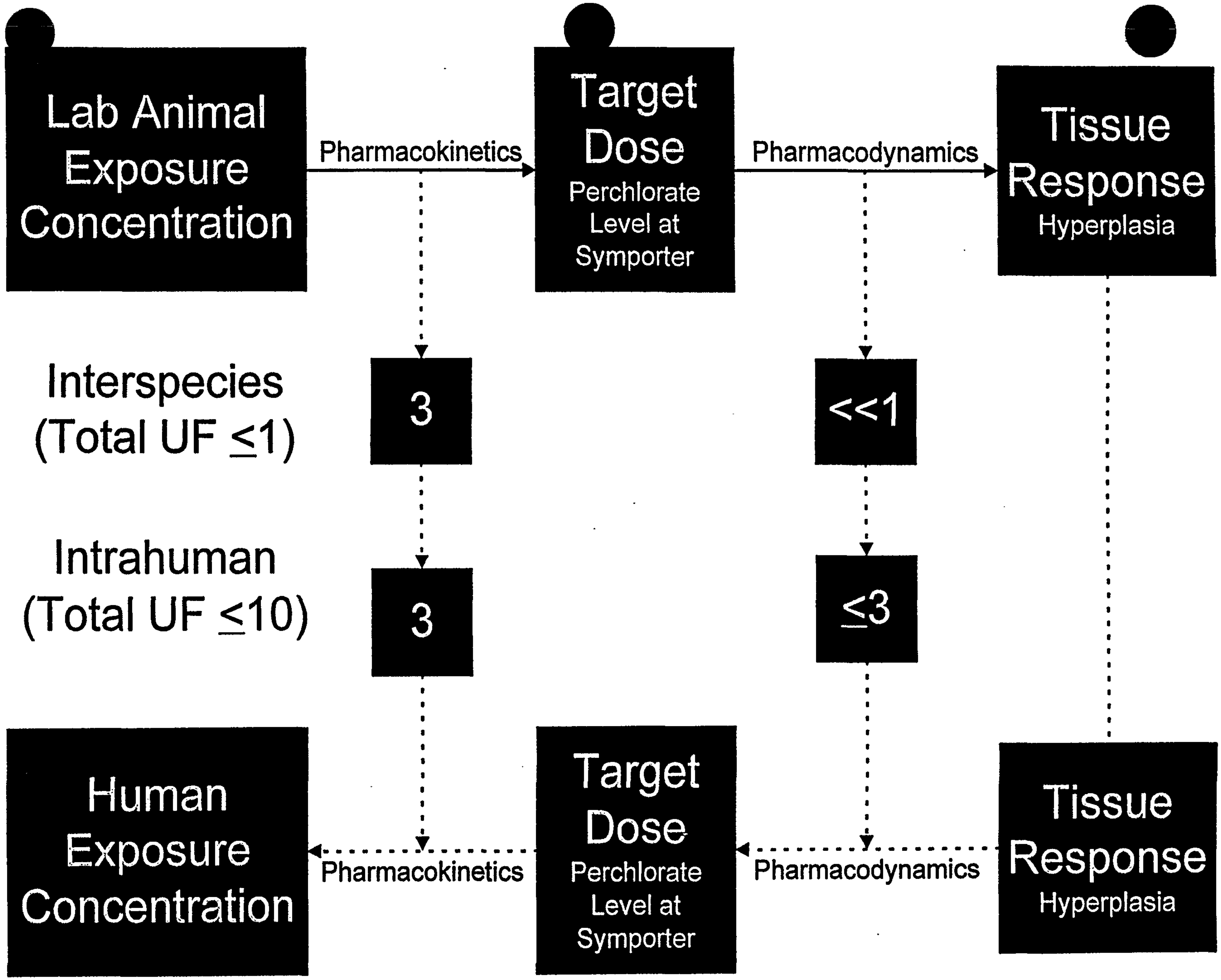
Barb[illegible]eck

Februa[illegible]0, 1999

San Bernardino, CA

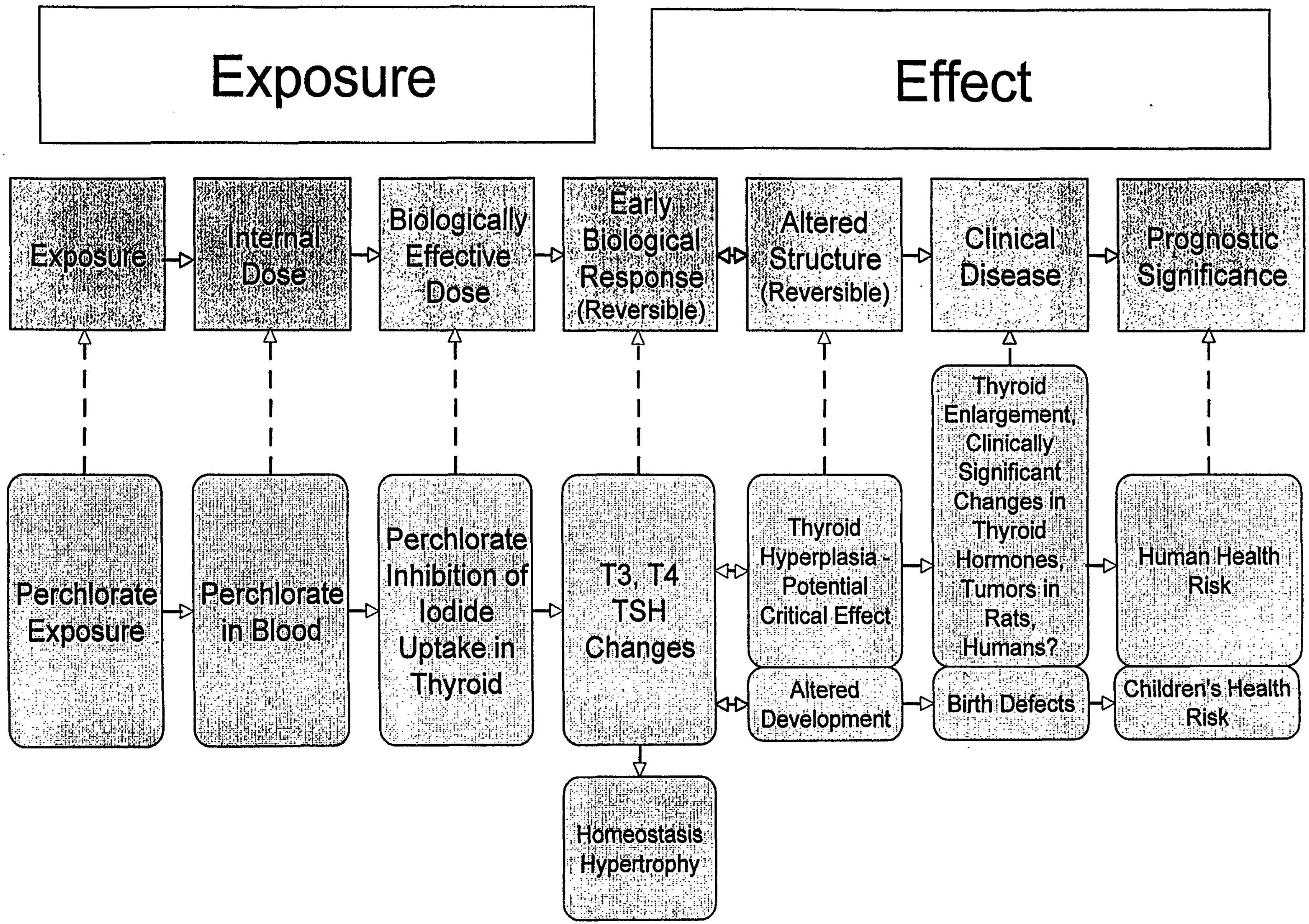
Gradient Corporation
An IT Company

Jellinek, Schwartz & Connolly, Inc.
An IT C[illegible]ay



Gradient Corporation

Jellinek, Schwartz & Connolly, Inc.


Exposure
Effect
Exposure
Internal Dose
Biologically Effective Dose
Early Biological Response (Reversible)
Altered Structure (Reversible)
Clinical Disease
Prognostic Significance
Perchlorate Exposure
Perchlorate in Blood
Perchlorate Inhibition of Iodide Uptake in Thyroid
T3, T4 TSH Changes
Thyroid Hyperplasia - Potential Critical Effect
Thyroid Enlargement, Clinically Significant Changes in Thyroid Hormones, Tumors in Rats, Humans?
Human Health Risk
Altered Development
Birth Defects
Children's Health Risk
Homeostasis Hypertrophy

Choice of the Critical Study and Critical Endpoint

- Rat neurodevelopment study:
  - PND5 data
  - Thyroid follicular-cell morphology changes at the lowest dose tested (0.1 mg/kg-day):
    - Increased cell height and/or diameter (*i.e.*, hypertrophy);
    - Decreased lumen size.
- Are the morphological changes merely histologically identifiable or are they indicative of a pathological process?

Physiological Significance of Follicular Cell Hypertrophy and Decreased Lumen Size

- Effects are reversible: No evidence for permanent cellular change found in rat pups exposed *in utero* and *via* breast milk to a maternal perchlorate dose as high as 10 mg/kg-day.
- No evidence offered by EPA/NCEA that such cellular changes, in and of themselves, fall outside the realm of physiologically normal thyroid morphology.

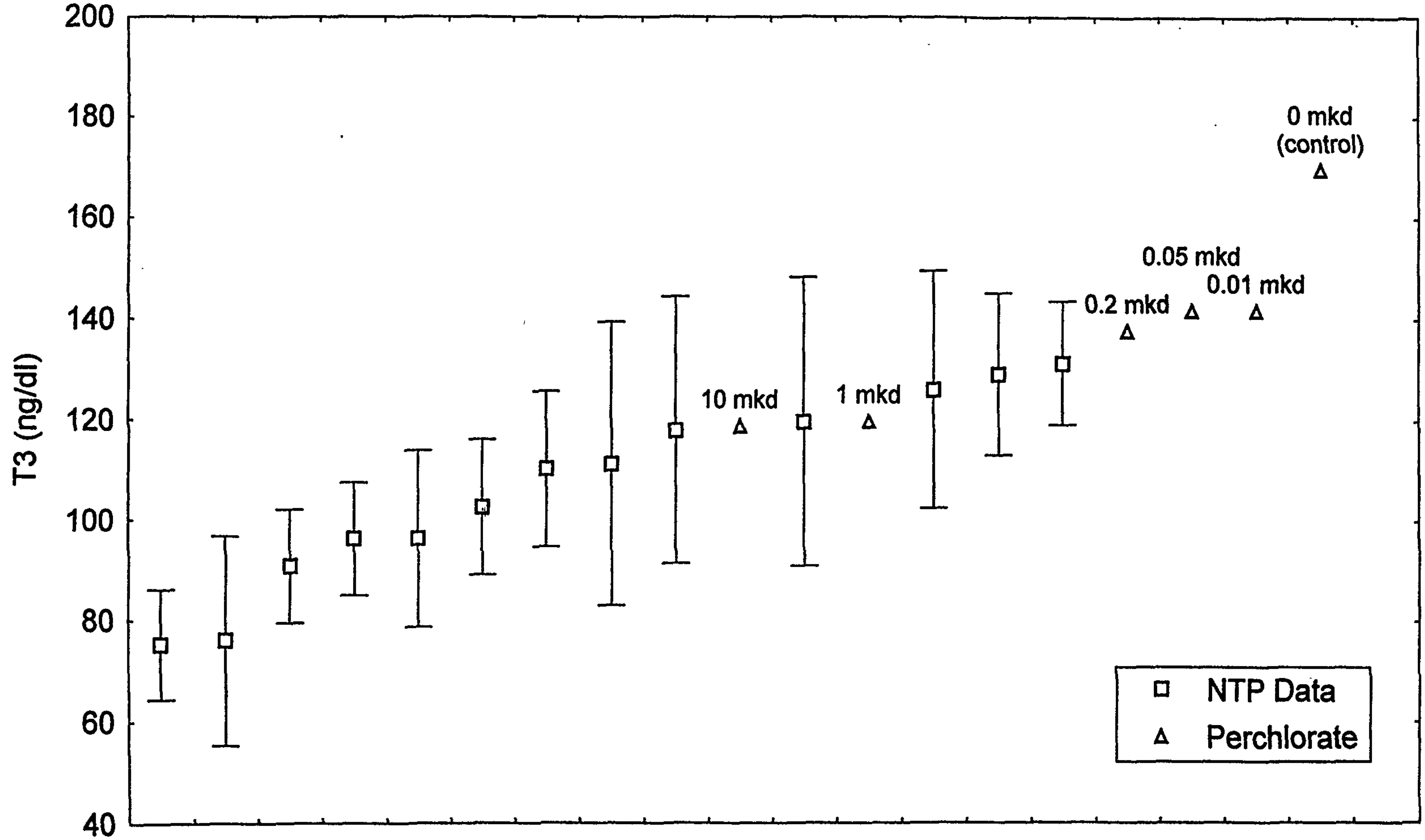
Table 1. Incidence Ratio of Any Evidence of Follicular-Epithelial-Cell Hypertrophy Among Rat Pups in the 1998 Neurodevelopmental Study of Perchlorate by Argus Laboratories, as Determined by Standard Histology

Time of Sacrifice	Control	Perchlorate Dose to the Dams (mg/kg-day)			
		0.10	1.0	3.0	10
PND5	0.25 (3/12)	0.67 (8/12)	0.75 (9/12)	0.67 (8/12)	1.00 (12/12)
PND10	0.40[a]	0.40[a]	0.40[a]	1.00[a]	1.00[a]
PND22[b]	0.52[a]	0.48[a]	0.68[a]	0.52[a]	0.48[a]

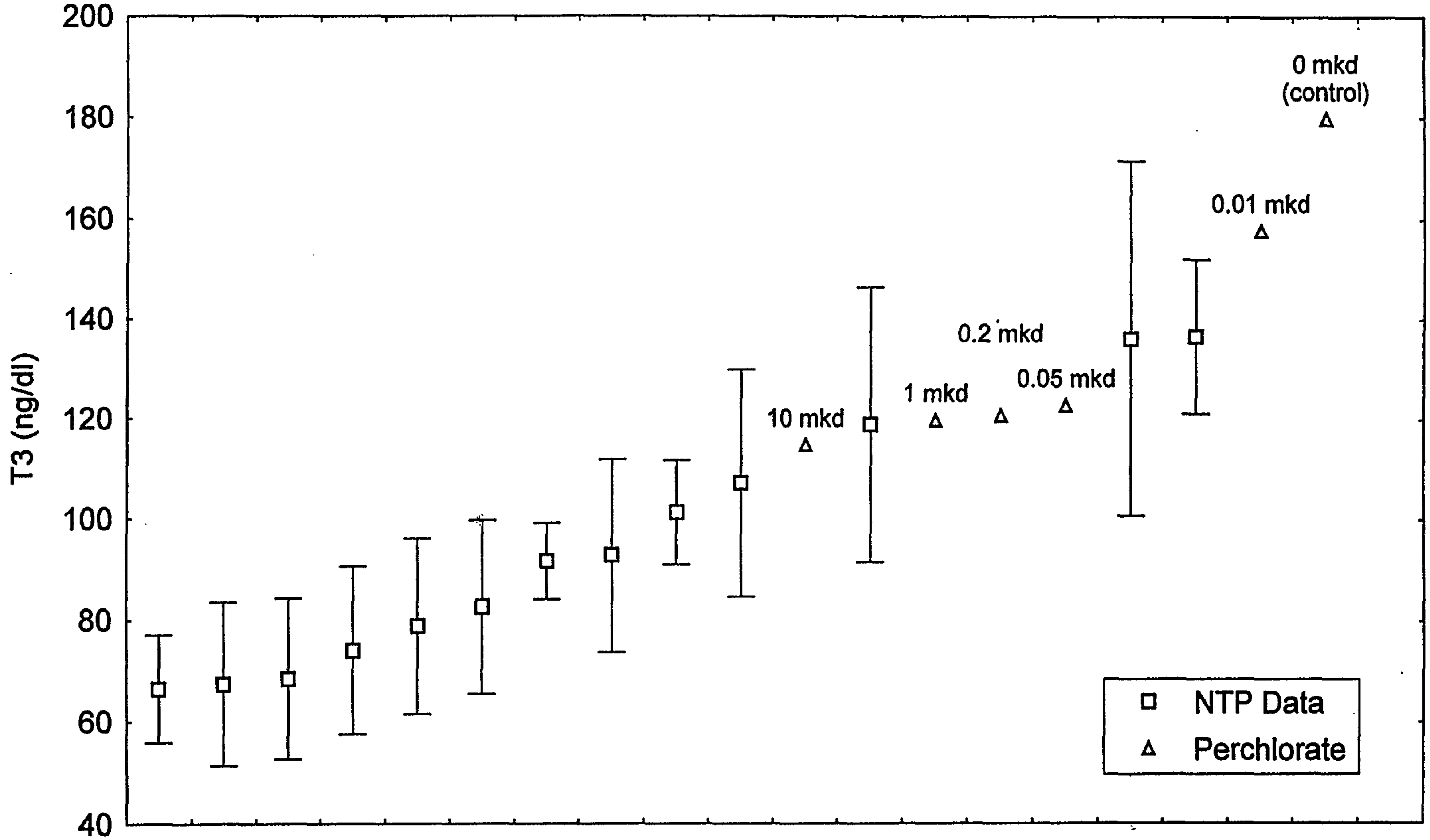
[a] The External Review Draft did not provide the absolute number of animals examined.

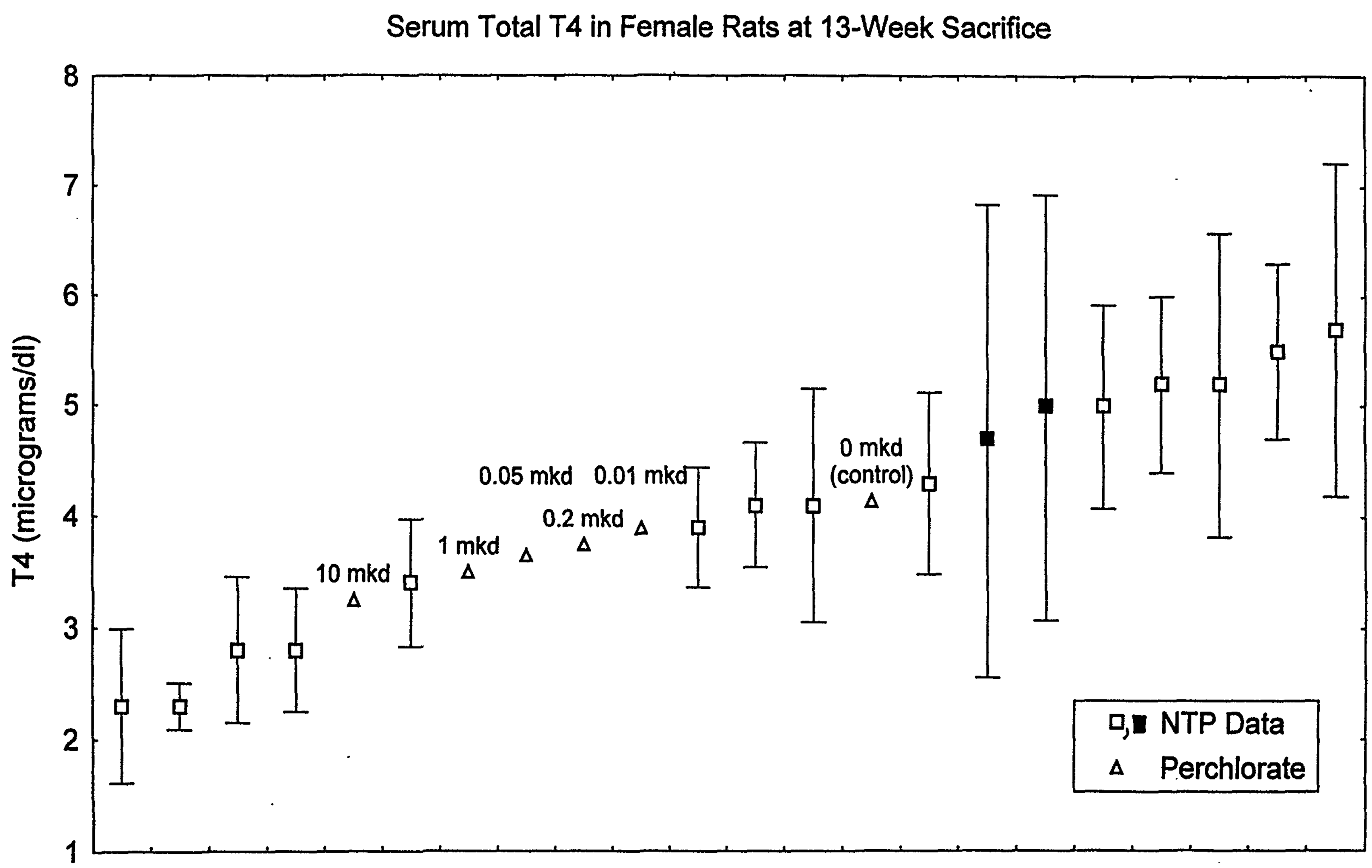
[b] Perchlorate exposure discontinued on PND10.

Serum Total T3 in Female Rats at 13-Week Sacrifice



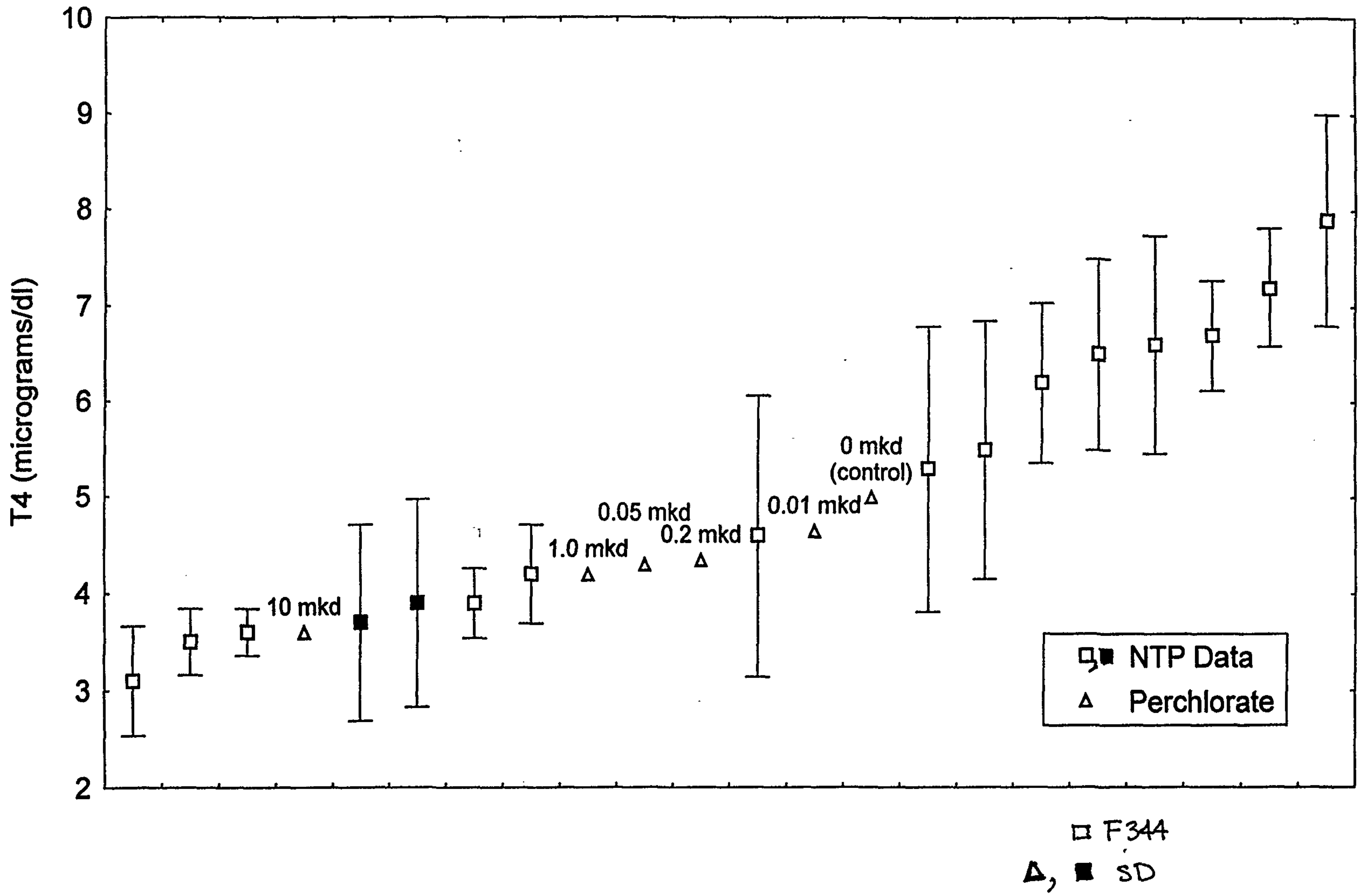
Serum Total T3 in Male Rats at 13-Week Sacrifice




Serum Total T4 in Female Rats at 13-Week Sacrifice
T4 (micrograms/dl)
8
7
6
5
4
3
2
1
10 mkd
1 mkd
0.05 mkd
0.2 mkd
0.01 mkd
0 mkd
(control)
NTP Data
Perchlorate

□ F344
△, ■ SD

Serum Total T4 in Male Rats at 13-Week Sacrifice



Correlation Analyses: EPA/NCEA Hypotheses

- Paired hormone comparisons
  - Expect positive correlations for T3 vs. T4;
  - Expect negative correlations for T3 vs. TSH;
  - Expect negative correlations for T4 vs. TSH.
- Paired hormone/histology comparisons
  - Expect positive correlations for TSH vs. histology;
  - Expect negative correlations for T3 vs. histology;
  - Expect negative correlations for T4 vs. histology.

Correlation Analyses

- In most cases, found statistically significant correlations of the expected sign.
- **Question:** Would the expected correlations be found if the high doses were removed from the analyses?
- **Answer:**
  - Not for T4 rank order vs. histology severity rating
  - Not for TSH rank order vs. histology severity rating
  - Not clear for T4 vs. TSH (evidence for and against)
  - Probably yes for T3 vs. T4.

Michael McClain
Jellinek, Schwartz and Connolly, Inc.

R. Michael McClain, PhD
Consultant in Toxicology
10 Powder Horn Terrace
Randolph NJ, 07859

February, 10, 1999

Ella Dardin
Research Triangle Institute
POB 12194
Research Triangle Park, NC 27709-2914

RE: *Comments to the External scientific Peer Review Panel for the document entitled "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information.*

I was asked by the firm Jellinek, Schwartz and Connolly, Inc. to review and provide a scientific evaluation of the EPA document on Perchlorate Environmental Contamination and to briefly comment to the Peer Review Panel on what I consider to be some of the more important issues in establishing the RfD based on the information in the document.

EPA has done a thorough job in compiling the available information on perchlorate and in evaluating the more recent information concerning the effects of perchlorate on thyroid gland function in the rat, reproductive performance and development, neurobehavioral and immunologic effects. The document represents a sound scientific approach for perchlorate risk assessment.

The primary, if not only effect, of perchlorate is on thyroid gland function. By virtue of its ability to competitively inhibit the uptake of iodine by the thyroid gland the expected responses to altered thyroid gland function are observed. These include decreased circulating levels of thyroid hormone T3 and T4, a compensatory increase in TSH and compensatory morphological changes in the thyroid gland including increased weight and follicular cell hypertrophy and hyperplasia.

Consistent diagnostic criteria needs to be used in the histologic evaluation of the thyroid gland.

The morphological effects on the thyroid gland are important for evaluating the effects of chemical treatment. In the various studies however, no consistent criteria are provided for the diagnosis of follicular cell hypertrophy and hyperplasia. For example, in the Caldwell studies hypertrophy/hyperplasia appears to be recorded as a single diagnosis, in the 90 day rat study only hyperplasia at the high dose of 10 mg/kg/day is recorded although the written description mentions hypertrophy. In the neonatal study the subjective histologic diagnosis of hypertrophy is not consistent with the morphometric measurements. Also, in the text of the report, the term hyperplasia is used in a few places when hypertrophy was reported. For a diagnosis using consistent criteria, the thyroid glands should be reevaluated by a pathologist well versed in the histology of the rat thyroid gland. This and any morphometric examinations should be performed on a randomized blind basis.

As discussed in the EPA document there are marked species differences in thyroid gland function. The rat has an active thyroid gland, a high turnover of thyroid hormone and very little reserve capacity. As compared to the primates and many other species the follicular epithelium of the control rat is hypertrophied and the diagnosis for the rat is hypertrophy beyond that, that already exists. There is a great deal of variation in the rat thyroid gland with respect to the height of the follicular epithelium. Because of this and the normal variation, not all pathologists will record hypertrophy until the thyroid is stimulated to the point that hyperplasia is also evident.

Follicular cell hyperplasia in the presence of TSH is a more appropriate endpoint in establishing the RfD.

Follicular cell hypertrophy is indicative of an increased functional activity of the thyroid gland and should be regarded as a functional adaptive change and as such should not be considered an adverse effect. The EPA has used hypertrophy to set the RfD; however in my opinion follicular cell hyperplasia in the presence of increased TSH would be a more appropriate endpoint. Although these are also adaptive changes, this could be considered to be a response to altered thyroid gland function to a degree that is considered adverse.

Follicular cell hypertrophy and hyperplasia are distinctly different cellular events.

The proper histological diagnosis of hypertrophy and hyperplasia is also important because these are distinctly different cellular events. Hypertrophy is an increase in cell size indicating an increased functional activity. Hyperplasia is the result of cell division. Although hypertrophy precedes hyperplasia on the dose response continuum, hypertrophy does not necessarily progress to hyperplasia or in other words, hypertrophy is not necessarily a percussor lesion to hyperplasia. Hypertrophy can exist alone at a certain level of stimulation without necessarily progressing to hyperplasia. At a higher level of stimulation, hyperplasia, as a result of increased cell proliferation, a different cellular response, will be observed in addition to hypertrophy.

Extrapolation of thyroid function data in rats to risks for humans.

Because of the marked species differences in thyroid gland function between the rat and primates, I agree with the EPA that the extrapolation from rat data is conservative. In other words, the rat is more likely to overestimate than underestimate the risk of perchlorate to humans. Although all the data are not yet in and it is possible that the situation may change if the thyroids are reevaluated, I believe that the estimates are conservative. As far as the assessment of thyroid function is concerned, the Springborn 90 day rat study is better conducted than any of the others and should be the study used for the RfD.

Nevertheless there are a lot of people potentially exposed and it will be a long time before the environmental conditions improve. For the future or next steps, I believe that studies should be conducted in the non-human primate to ensure that the estimate of margin of safety is accurate. Also since potassium perchlorate is used as a human drug, I would explore the possibility of studies in humans at **low doses** (i.e. doses in the range of 1/10 to 1/100 the current clinically used doses for treatment of thyrotoxicosis in patients treated with the iodine containing antiarrhythmic agent amiodarone).

I appreciate the opportunity to comment on the EPA perchlorate document.

RICHARD PLEUS
INTERTOX

U.S. EPA's Failure to Consider Human Studies in the Provisional Development of the Perchlorate RfD

Richard C. Pleus, Ph.D.
INTERTOX
Seattle

Issues

- There is concern that the rat is more sensitive than the human to the effects of perchlorate.
- Clinical studies in humans are currently in progress; these will provide critical information regarding the dose-response for the effect of perchlorate on human thyroid function.
- In its December 31, 1998 document, U.S. EPA did not consider or even anticipate using human data.
- The release of the provisional RfD was driven by meeting a schedule rather than the pursuit of good science.

Did US EPA consider human studies in the development of the provisional RfD?

- The EPA document does not consider human data of any kind in the derivation of the RfD for perchlorate.
- Perchlorate was used as a therapeutic agent in the 1950s; there have been several clinical and occupational studies on the health effects of perchlorate.
- Two human studies are currently in progress; these could be important for
  - determining the dose-response for effects on thyroid hormones;
  - determining interspecies differences in sensitivity to the thyroidal and extrathyroidal actions of perchlorate, including any effects on hematology and blood chemistry.

Protocol of Braverman *et al.*

- Perchlorate given to 8 adult, male volunteers at a dose of 10 mg/day (~ 0.14 mg/kg/day);
- 14-day exposure;
- Four or five volunteers have completed the perchlorate exposure and iodide-uptake tests;
- Follow-up examinations for all volunteers to be completed by March 1, 1999.

Protocol of Brabant *et al.*

- Perchlorate given to seven adult volunteers per dose;
- Doses of 7, 70, or 840 mg/day (~ 0.1, 1, or 12 mg/kg/day, respectively);
- 14-day exposure;
- Study was expected to start January 1999;
- No results of perchlorate exposure at this time.

CONCLUSION

- Human studies currently in progress will provide dose-response information for the known thyroidal effects of perchlorate and any additional effects on blood chemistry and hematology.
- Data from the new human studies will provide information useful to the comparison of perchlorate sensitivity in laboratory animals and humans.
- U.S. EPA has not considered or anticipated using human data; rather, an enforced timetable has been driving the release of a provisional RfD.
- Let good science prevail.

Appendix I

Introductory Presentations by EPA at the Workshop

INTRODUCTORY PRESENTATIONS BY EPA

To begin the workshop, EPA presented background material on the perchlorate issue and risk assessment. Peter Grevatt, of EPA's Office of Solid Waste and Emergency Response, presented a brief overview of the perchlorate peer review process. This was followed by Kevin Mayer of EPA's Region IX who presented a local perspective on the perchlorate issue, including the history of the issue and areas of the country with perchlorate releases. William Farland of EPA's National Center for Environmental Assessment (NCEA) discussed the risk assessment process for perchlorate, including the development of a revised Reference Dose (RfD) and comprehensive characterization. Annie Jarabek, also of EPA's NCEA, presented a summary of EPA's mode-of-action approach to human and ecological risk assessment for perchlorate.

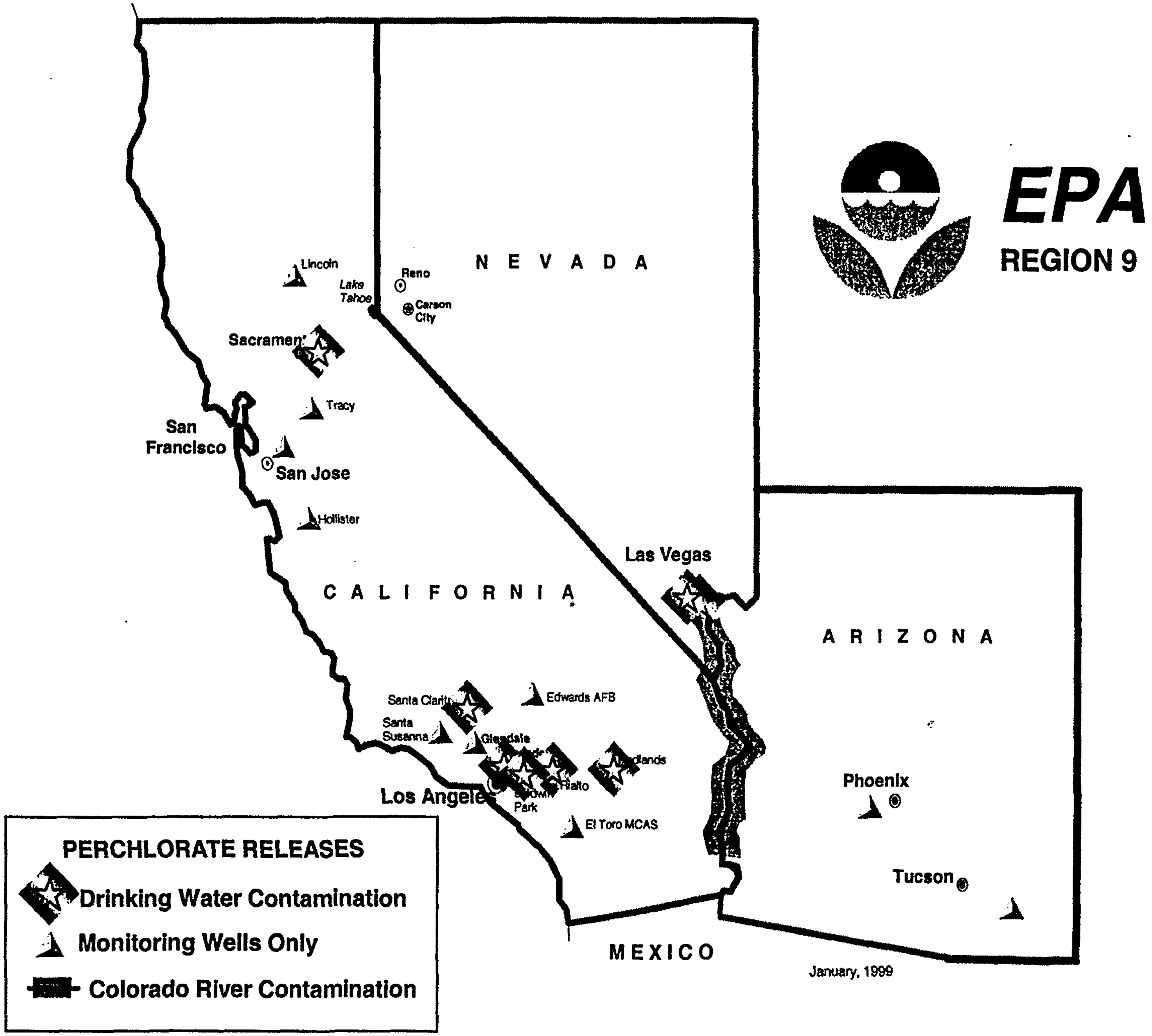
Kevin Mayer
EPA, Region 9

PERCHLORATE

WELCOME to Region 9

Kevin Mayer
Superfund Program
U.S. EPA, Region 9


EPA
REGION 9
NEVADA
Reno
Carson City
Lake Tahoe
Sacramento
San Francisco
San Jose
CALIFORNIA
Las Vegas
ARIZONA
Phoenix
Tucson
Los Angeles
San Diego
HAWAII
MEXICO



EPA
REGION 9
NEVADA
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Carson City
Lincoln
Lake Tahoe
Sacramen
Tracy
San Francisco
San Jose
Hollister
CALIFORNIA
Las Vegas
ARIZONA
Santa Clarit
Edwards AFB
Santa Susanna
Glendale
dlands
Rialto
Park
Los Angele
El Toro MCAS
Phoenix
Tucson
MEXICO
January, 1999
PERCHLORATE RELEASES
Drinking Water Contamination
Monitoring Wells Only
Colorado River Contamination

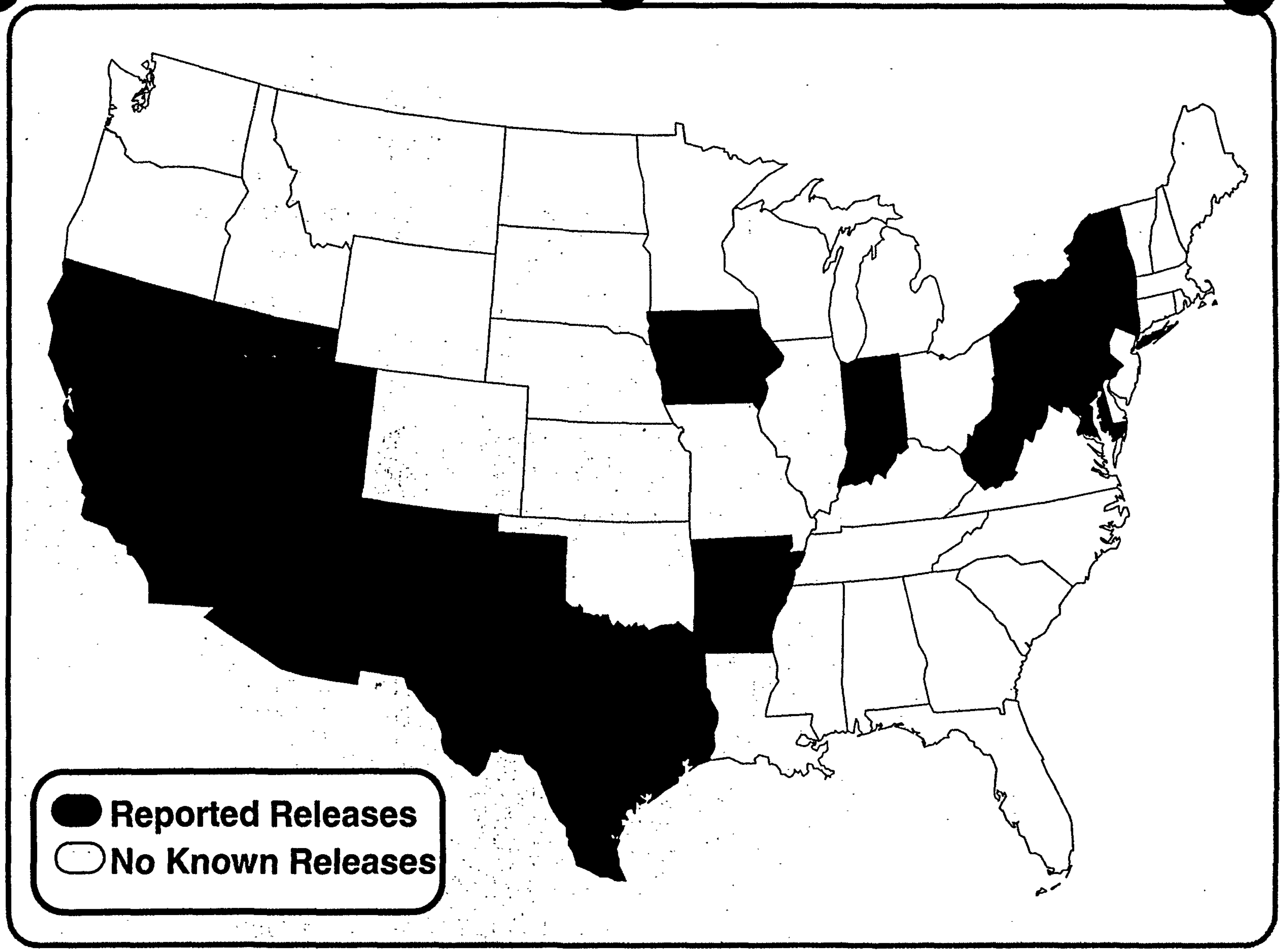
HISTORY - Before 1997

- 1980s - Aware of Perchlorate in CA, NV
- 1985-86 - San Gabriel Valley
- 1990s - Rancho Cordova (ppm)
- 1992-95 - Provisional Reference Dose (ppb range)
- 1997 - Analytical breakthrough

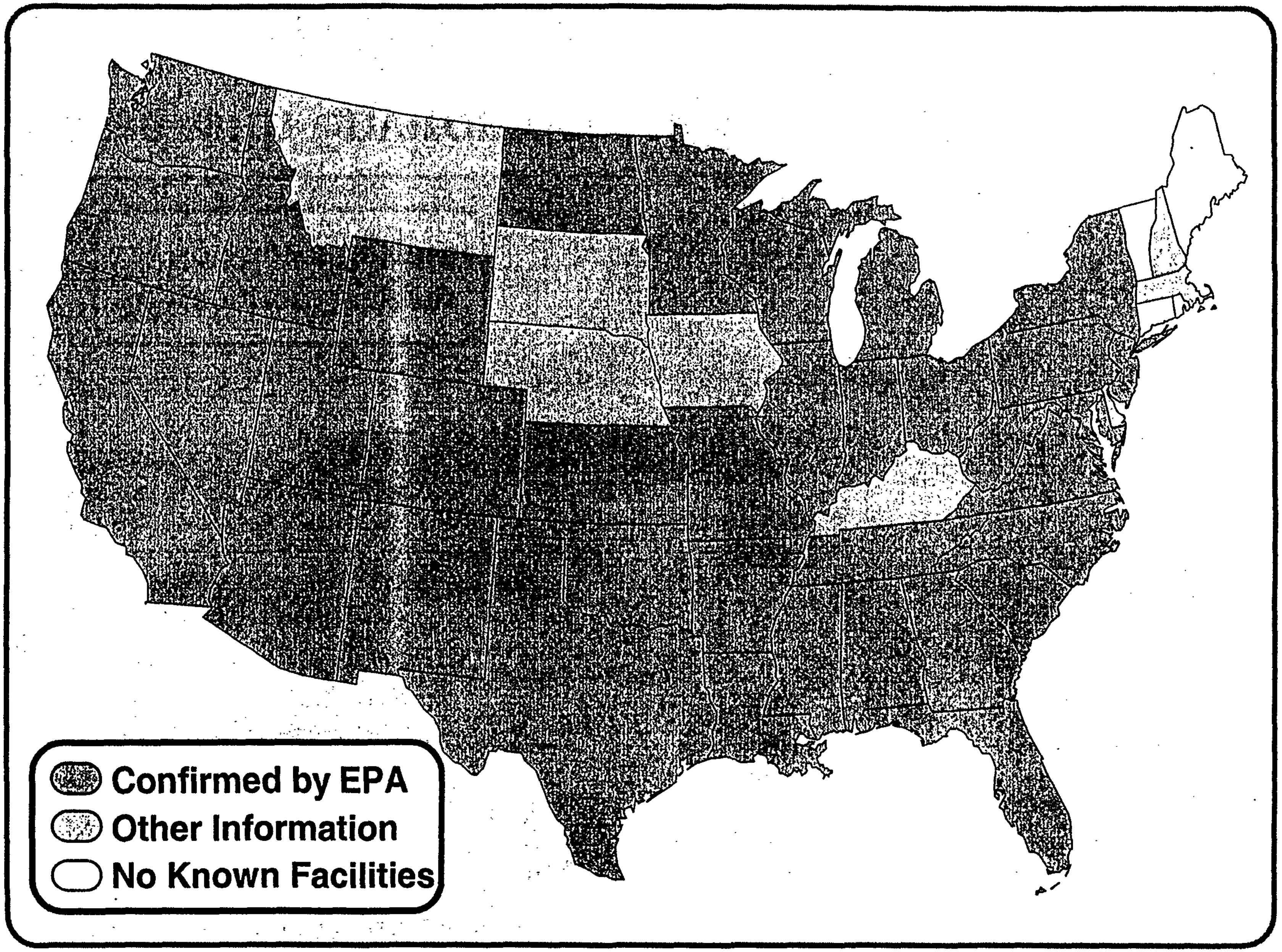
Agency for Toxic Substances and Disease Registry - ATSDR (January 21, 1986):

"...Given the proprietary nature of the laboratory method for quantification and the poor quality assurance results noted, the **data do not prove that perchlorate ion has actually been found.** If the presence of perchlorate ion is confirmed, the **scientific database** on this ion is **insufficient to generate either an acute or longer-term health advisory for drinking water**"

"... The minimal acute toxicity data available suggest that one or two ppm perchlorate ion would not represent an immediately acute and substantial threat to the public health. The ATSDR does not consider this level to be "safe" in the absence of experimental data.."



States with Environmental Releases of Perchlorate



States with Perchlorate Manufacturers or Users

Example Sites

- Southern CA - All 50 wells - Avg 25ppb, Range 4 ppb-130 ppb, std dev = 27
- River location - Avg 6 ppb, Range 4-9 ppb
- Northern CA - 11 Public Water Supply wells (in 1997), Avg 125 ppb, Median 93, Range <4 ppb to 340 ppb, std dev = 125
- Southern CA - All 37 wells - Avg 126, Median 34 ppb, Range 4 to 1,100 ppb, std dev = 239

William Farland
EPA, NCEA

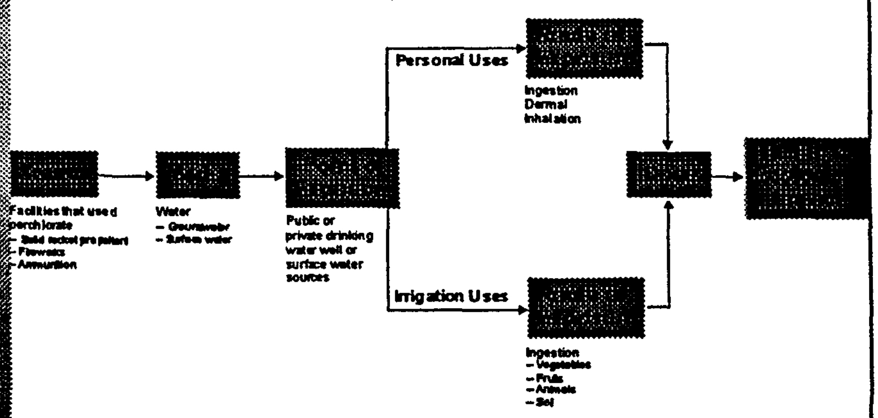
The Perchlorate Environmental Contamination Challenge: EPA ORD Assessment Strategy

William H. Farland, Ph.D.
Director
National Center for Environmental Assessment
U.S. EPA

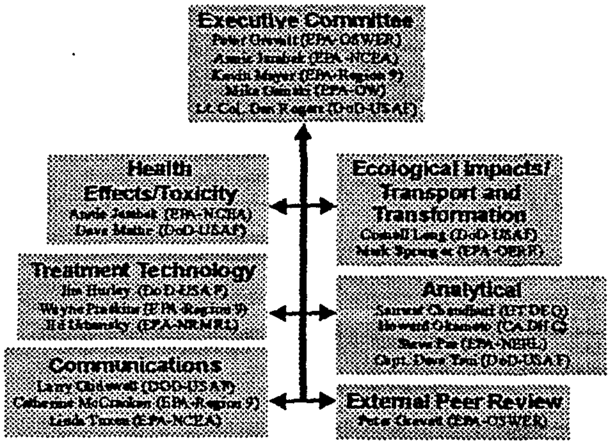
Perchlorate Peer Review Workshop
San Bernardino, California
February 10-11, 1999

I P S C

Comprehensive Characterization of Perchlorate Contamination



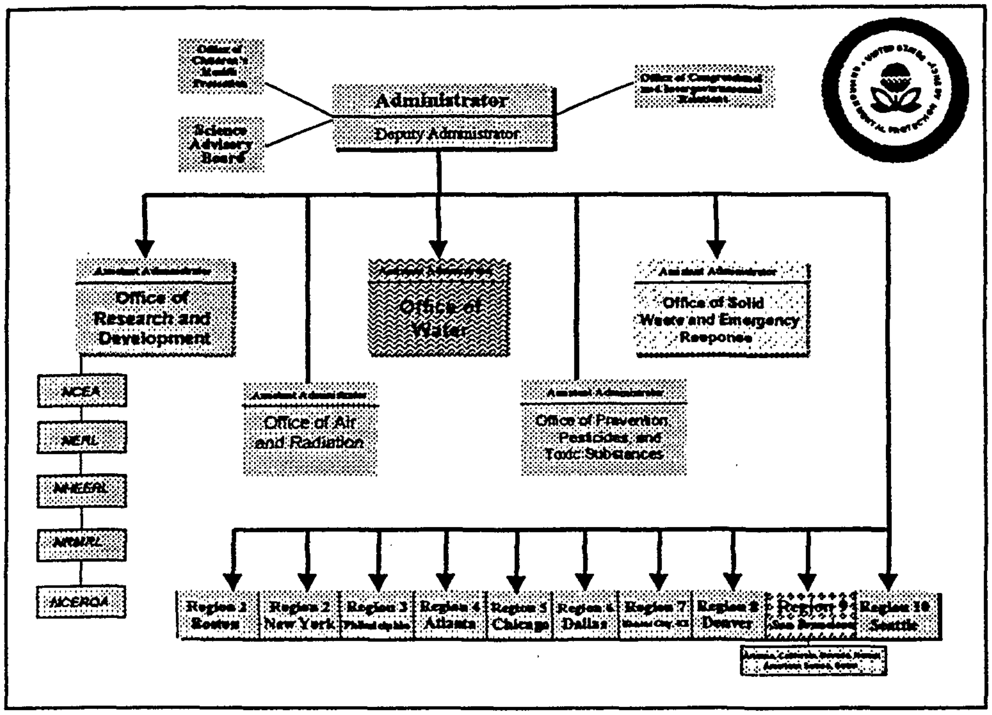
Interagency Perchlorate Steering Committee (IPSC)



The Perchlorate Contamination Challenge

Pro-Active Partnership

- Unprecedented timeframe
- Targeted expertise
- Commitment to continued improvement



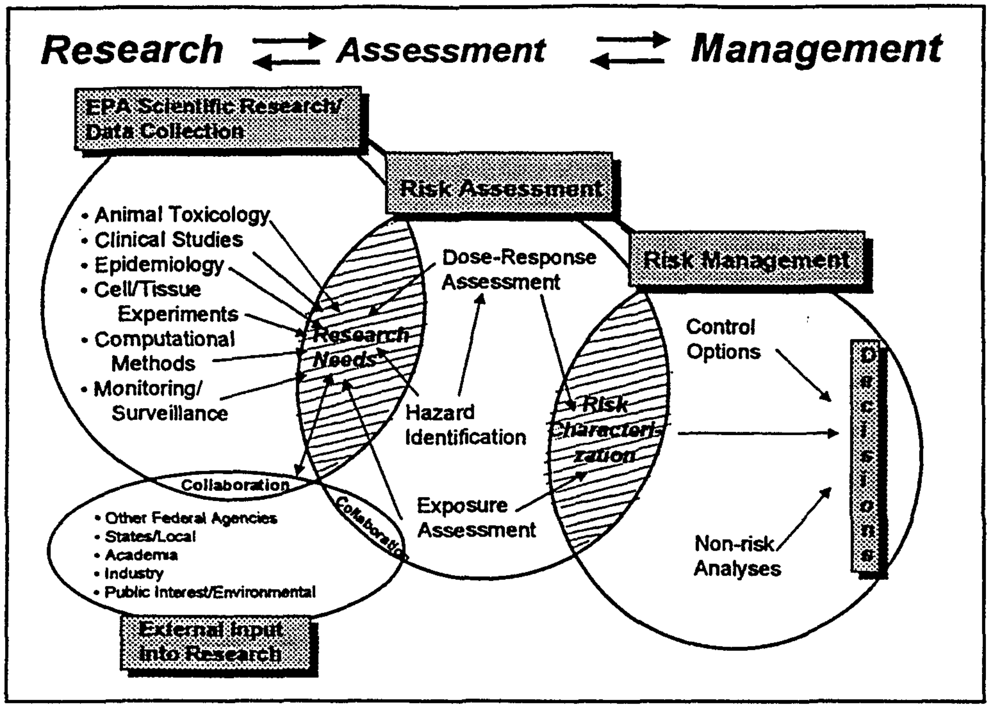
The Perchlorate Contamination Challenge

Credible Science

↓

Credible Decisions

- Accurate risk characterization
- Appropriate management strategies



Recent Emphasis Focuses on the Development and Use of Better Data

"The quality of risk analysis will improve as the quality of input improves. As we learn more about biology, chemistry, physics, and demography, we can make progressively better assessments of the risks involved. Risk assessment evolves continually, with re-evaluation as new models and data become available."

"Science and Judgment in Risk Assessment" (National Research Council, 1994)

The Perchlorate Contamination Challenge:

An Integrated Approach

- Occurrence survey
- Stakeholder issues
- Health effects / toxicology
- Analytical methods (Detection Limit)
- Ecological impact / transport & transformation
- Treatment technology
- Technology transfer

Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information

State-of-the-Science

- Human Health Assessment Based on Recent EPA Guidelines
- Harmonized Approach to Noncancer and Cancer Toxicity Based on Mode of Action
- Ecological Assessment Based on Recent EPA Framework
- Risk Assessment Recognized as an Iterative Process
- Internal and External Peer Review

Required for All Recommended Studies

- Good Laboratory Practice Standards EPA (40 CFR Part 792)
- Animal housing and care based on Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and Guide for the Use of Laboratory Animals (NIH Publication No. 96-03, 1996)

Standard Operating Procedures

- Protocol review by expert panel
- EPA testing guidelines
- Standardized QA / QC process

EPA Risk Assessment Guidelines

- Principles and procedures to frame the conduct of risk assessments
- Promote consistency and technical quality of scientific inferences
- Flexible, full consideration to all relevant scientific information case-by-case
- Revised as experience and scientific consensus evolve

EPA Risk Assessment Guidelines

- **Developmental toxicity (1991)**
  FR 56(234): 63798 - 63826
- **Proposed guidelines for carcinogen risk assessment (1996)**
  FR 61: 17960-18011.
- **Reproductive toxicity (1997)**
  EPA No. EPA/630/R-96/009a
  NTIS PB97-100093
- **Neurotoxicity (1998)**
  EPA No. EPA/630/R-95/001Fa
  NTIS PB98-117831
- **Thyroid follicular cell tumors (1998)**
  EPA/630/R-97-002

Technical Hazard Characterization

- Integrates the data analysis of all relevant studies into weight-of-evidence conclusions
- Presents the exposure conditions (route, duration, pattern, magnitude) under which effects are expressed
- Presents the agent's mode of action

Mode of Action Provides Important Insight to Characterization of Toxicity

- A chemical's influence on the molecular, cellular, and physiological functions in producing tumors
- Helps interpret the relevancy of experimental animal data
- Guides choice of appropriate dose-response procedure (linear, non-linear, mixed)
- Platform to harmonize approaches to cancer and noncancer toxicity

Toxicity Study Review and Revised Harmonized "RfD" Assessment

- Review of existing and new toxicity data in experimental animals and humans
- Hazard identification
- Dose-response evaluation
  - Noncancer
    - Designation of effect levels (mathematical modeling or NOAEL / LOAEL procedure)
    - UF assignment
    - Uncertainty characterization - confidence statements
  - Cancer
    - Genotoxic or indirect

Revised Harmonized Oral Human Health Benchmark ("RfD")

- Data across comprehensive array of endpoints to establish target tissue
- Mechanistically-motivated special studies to characterize critical dose-response relationships
- Harmonized nonlinear approach to both cancer and noncancer assessment based on mode of action
- Future refinements as required by new data

Screening Ecological Assessment

- Limited in scope due to the set of data that could be generated in short time frame
- Scope nevertheless responsive to stakeholder concerns regarding lettuce
- Problem formulation focused on selection of assessment endpoints, derivation of conceptual model, and analysis
- Analysis revealed uncertainties and research needs identified to guide next tier of testing

Assessment Development Process

- Internal peer review (December 1998)
- External peer review (February1999)
- Review of additional pending data
- Response / revisions subsequent to external peer review
- Additional external peer review
- Submit final revised assessment to Integrated Risk Information System (IRIS) process

Risk Assessment is an Iterative Process

Continued Improvement

- Provisional RfD (1992, 1995) - Superfund Technical Support Center, NCEA-Cin
- Revised Harmonized Human Oral Benchmark "RfD" (December 1998) - NCEA
- External Peer Review (1999)
- IRIS Consensus Review
- Refinements as required in the future

Comprehensive Characterization

- Health and ecotoxicology assessment required as focal points of integrated approach
- Contemporary with progress in other areas
- Risk characterization precluded by LACK OF accurate exposure surveys
- Identification of research needs and recommendations will improve path forward

Annie Jarabek
EPA, NCEA

Perchlorate: Mode-of-Action Approach to Human and Ecological Assessment

Annie M. Jarabek
National Center for Environmental Assessment
U.S. Environmental Protection Agency

Perchlorate Peer Review Workshop
San Bernardino, California
February 10-11, 1999

I P S C

EPA Perchlorate Risk Assessment Team

- **Randy Bruins** (NCEA Cin) Ecotoxicology
- **Harlal Choudhury** (NCEA Cin) Genl tox / risk assess
- **Eric Clegg** (NCEA W) Reproductive toxicology
- **Kevin Crofton** (NHEERL) Neurotoxicology
- **Vicki Dellarco** (OW) Genetic toxicology
- **Andrew Geller** (NHEERL) Neurotoxicology
- **Annie Jarabek** (NCEA RTP) Dosimetry / risk assess
- **Gary Kimmel** (NCEA W) Developmental toxicology
- **Mary Manibusan** (OW) Genl tox / risk assess
- **Ralph Smialowicz** (NHEERL) Immunotoxicology
- **Glenn Suter** (NCEA Cin) Ecotoxicology

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- Brooks AFB (and its contracted laboratories): Cornell Long and Ron Porter
- Perchlorate Study Group (PSG) [and its contracted laboratories and consultants]: Mike Girard, TERA

Unique Attributes

- Partnership to develop data base
- Both human and ecological risk assessments of available data
- Harmonized approach to noncancer and cancer toxicity based on mode of action

Outline

- Human Health Assessment
  - Derivation Procedures
  - Mode of Action
  - Development of testing strategy
  - Results, issues, research needs
- Ecological Screening Assessment
  - Approach
  - Results, issues, research needs

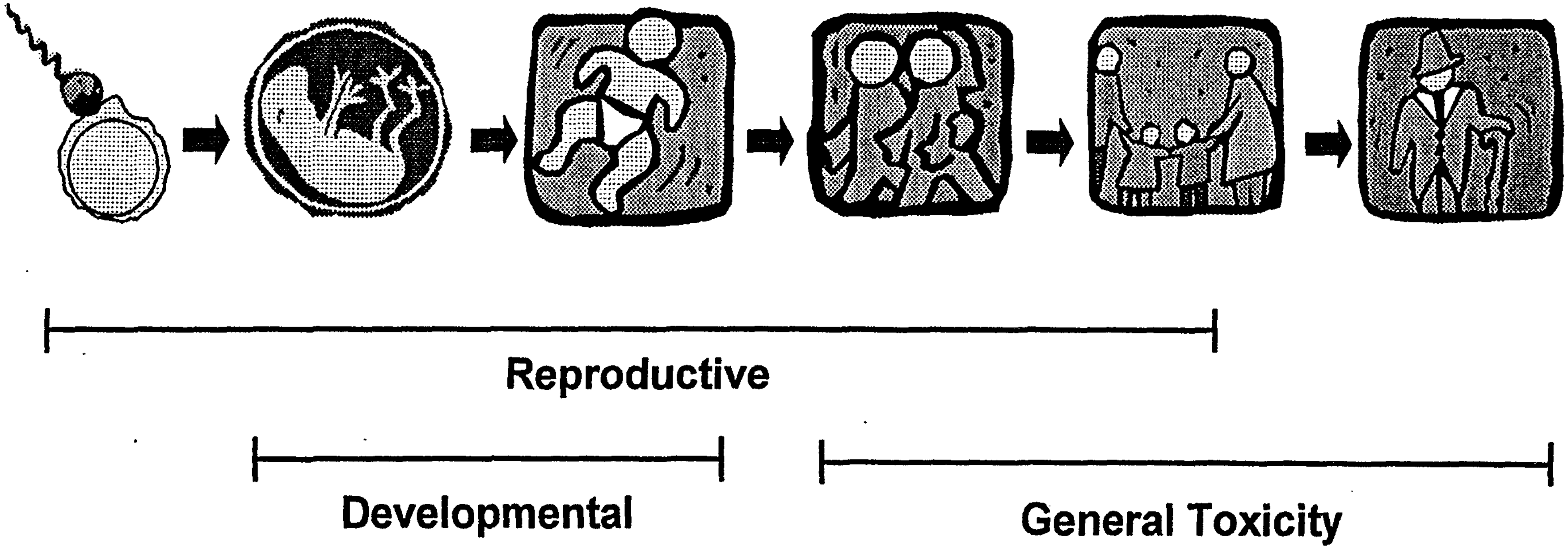
Outline

- Presentation of Data Received since December 1998
  - 1. Completed analyses
  - 2. Preliminary analyses
- Presentation of Ongoing Studies
  - 3. Pending data

Definition

An oral reference dose (RfD) is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer health effects during a lifetime.

A High Confidence RfD is Based on Data that Addresses All Potentially Critical Life Stages.



Minimum Data Base for Derivation of an RfD

Mammalian Data Base**	Confidence	Comments
A. Two Chronic Oral Bioassays in Different Species B. One 2-Generation Reproductive Study C. Two Developmental Toxicity Studies in Different Species	High*	Minimum Data Base for High Confidence
One Subchronic Oral Bioassay	Low	Minimum Data Base for Estimation of an RfD

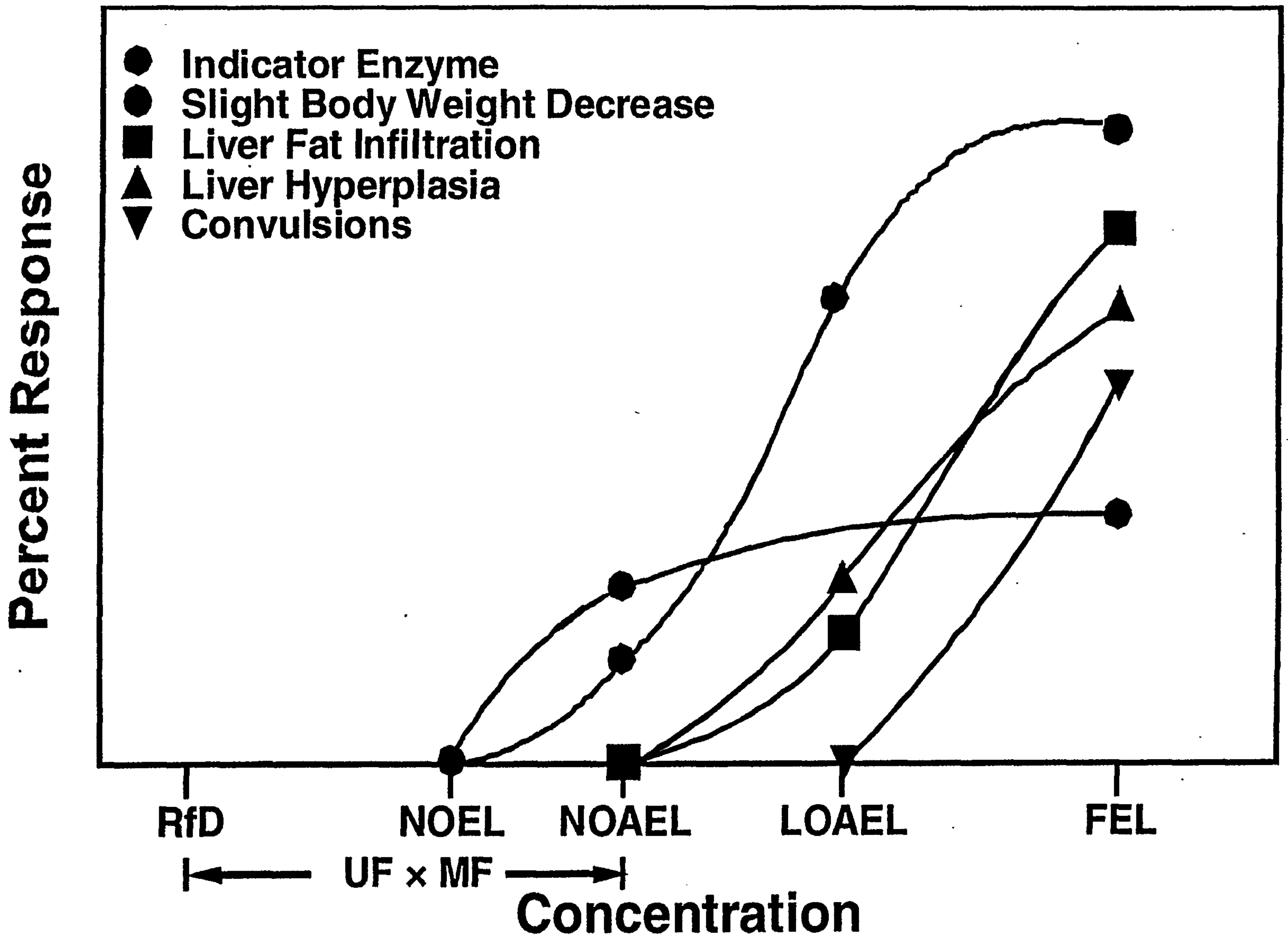
* Rationale is to address all potentially critical life stages

** Rationale is to use different species to evaluate variability in species sensitivity unless a particular laboratory animal model is more appropriate

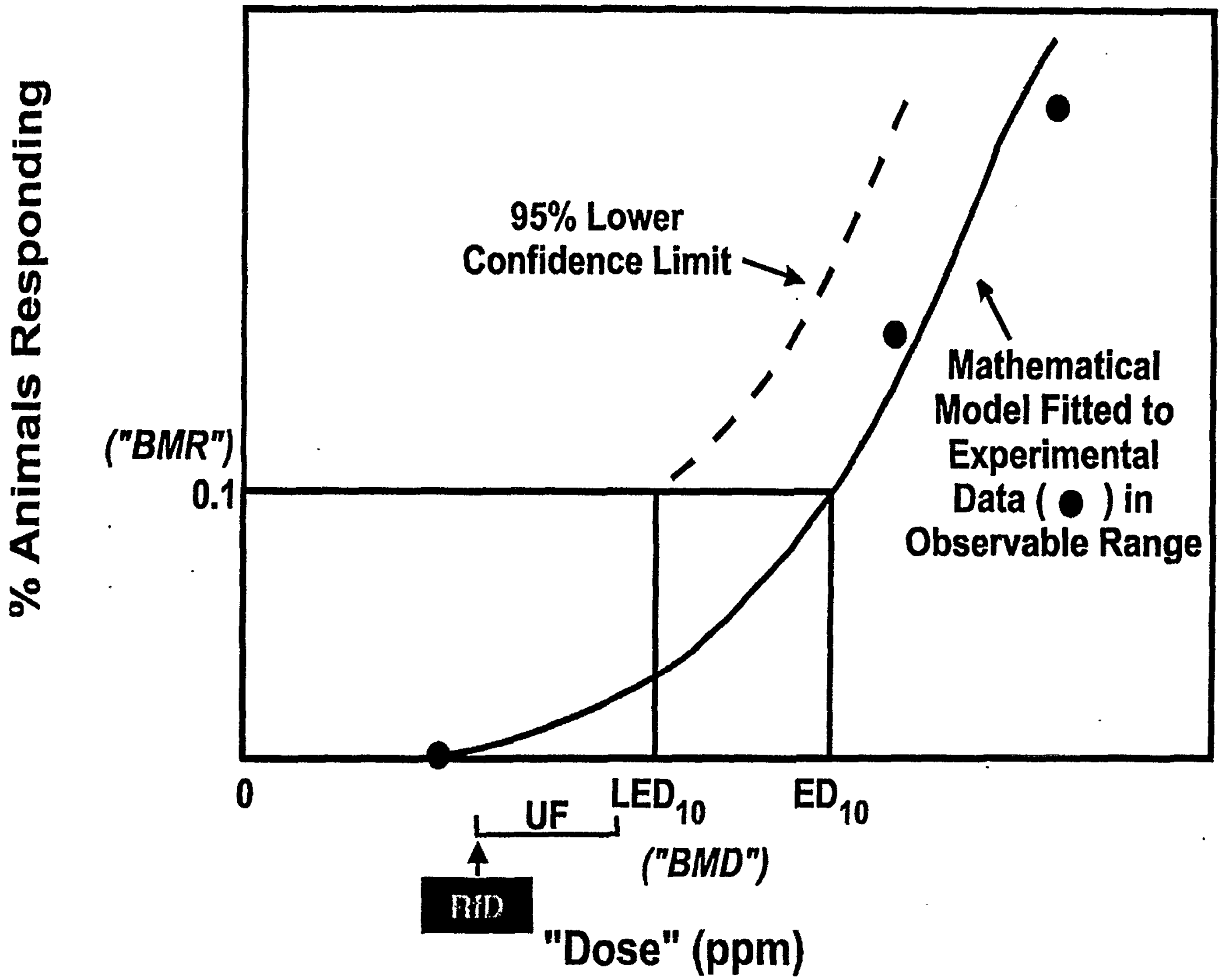
RfD Derivation

- Hazard identification and data array analysis
- Designation of effect levels (NOAEL, BMD)
- Choice of critical effect
- Dosimetric adjustment
- Application of uncertainty factors (UF)
- Characterization of uncertainty (confidence levels)

Data Array and Oral Reference Dose (RfD) Derivation



"Benchmark Dose" Approach to Dose-Response Analysis for Noncancer Endpoints



$$RfD = \frac{NOAEL^{*}[HED]}{UF \times MF}$$

Where:

NOAEL*[HED] =

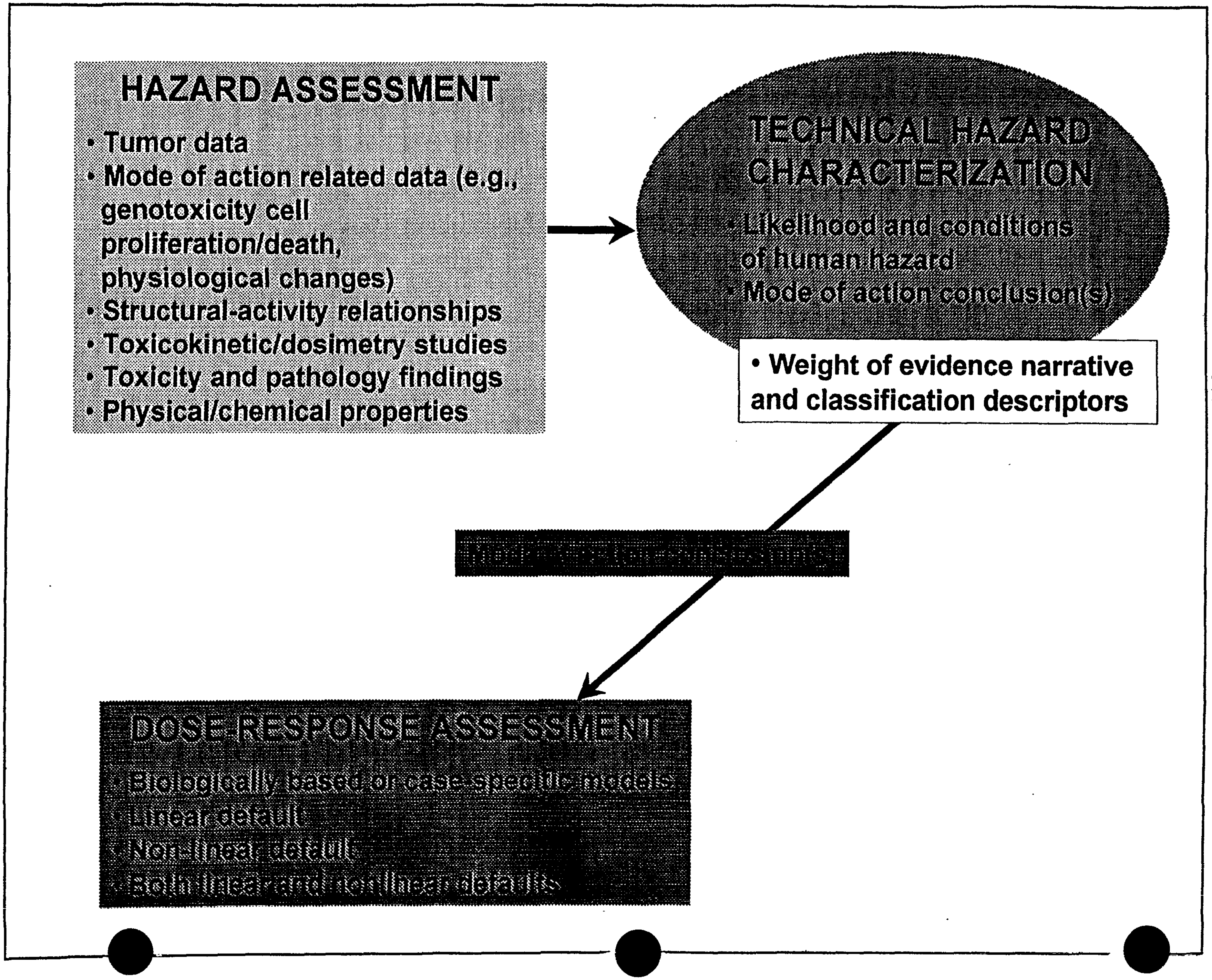
The NOAEL or equivalent effect level obtained with an alternate approach (*), dosimetrically-adjusted to a human equivalent dose [HED].

UF =

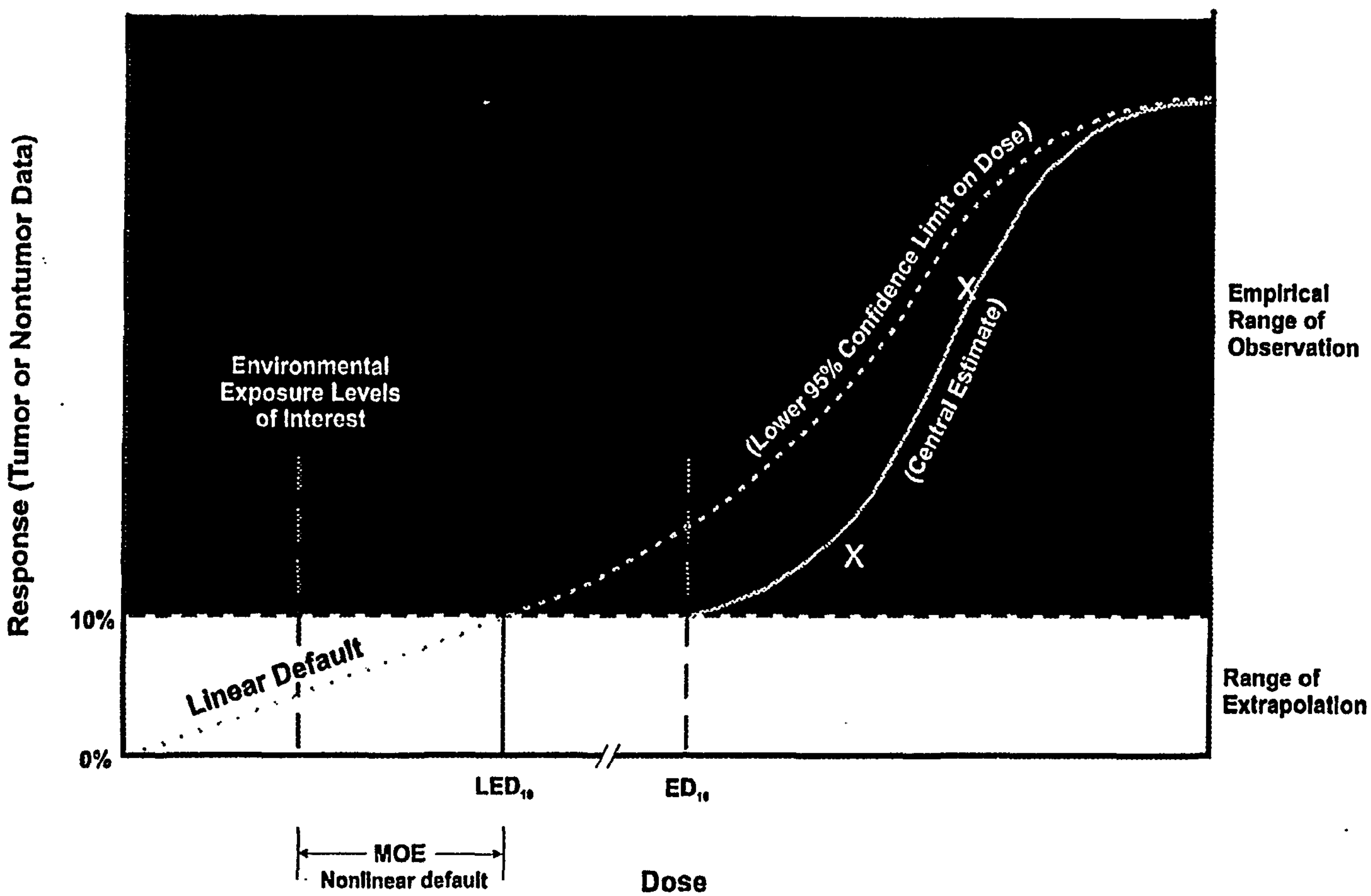
Uncertainty factor(s) applied to account for the extrapolation required from the characteristics of the experimental regimen to the assumed human scenario, and

MF =

Modifying factor to account for scientific uncertainties in the study(ies) chosen as the basis for the operational derivation, e.g., poor statistical power or exposure characterization.


HAZARD ASSESSMENT
• Tumor data
• Mode of action related data (e.g., genotoxicity cell proliferation/death, physiological changes)
• Structural-activity relationships
• Toxicokinetic/dosimetry studies
• Toxicity and pathology findings
• Physical/chemical properties
TECHNICAL HAZARD CHARACTERIZATION
• Likelihood and conditions of human hazard
• Mode of action conclusion(s)
• Weight of evidence narrative and classification descriptors
DOSE-RESPONSE ASSESSMENT
• Biologically based or case specific models
• Linear default
• Non-linear default
• Both linear and nonlinear defaults

Proposed EPA Cancer Guidelines
Extrapolation Approaches Based on Mode of Action



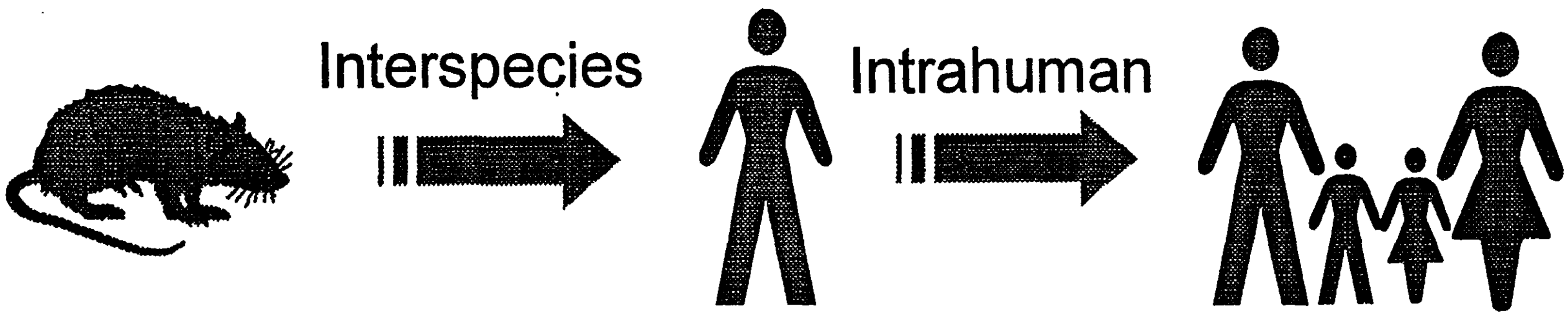
Factors for Uncertainties in Applied Extrapolations

10_H	Human to Sensitive Human
10_A	Experimental Animal to Human
10_S	Subchronic to Chronic Duration
10_L	LOAEL(HEC) to NOAEL(HEC)
10_D	Incomplete to Complete Data Base

Modifying Factor

MF Professional Assessment of Scientific Uncertainties of the Study and Data Base not Explicitly Addressed Above. Default for the MF is 1.0 e.g., applied for small sample size or poor exposure characterization.

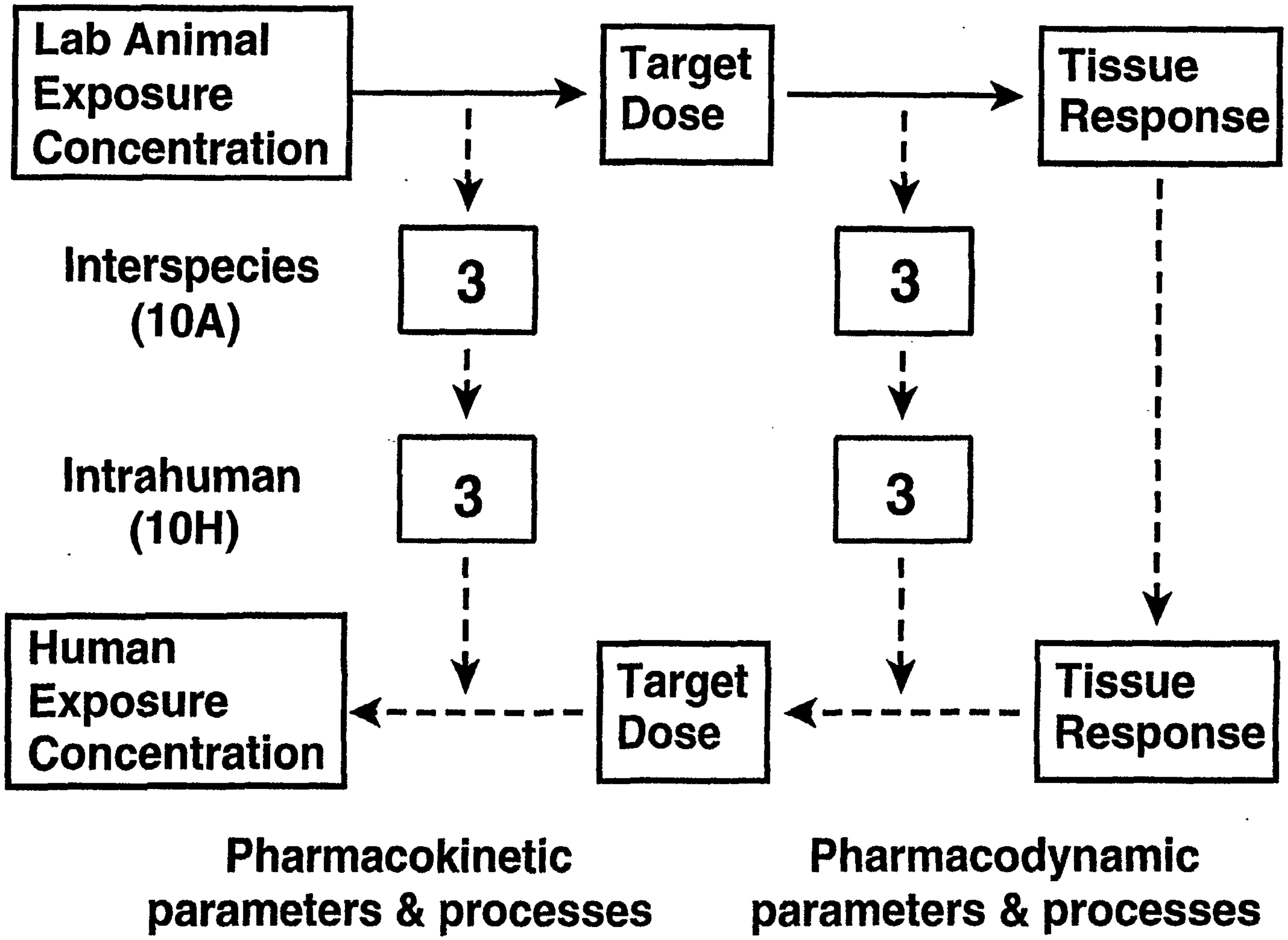
Extrapolation Uncertainties



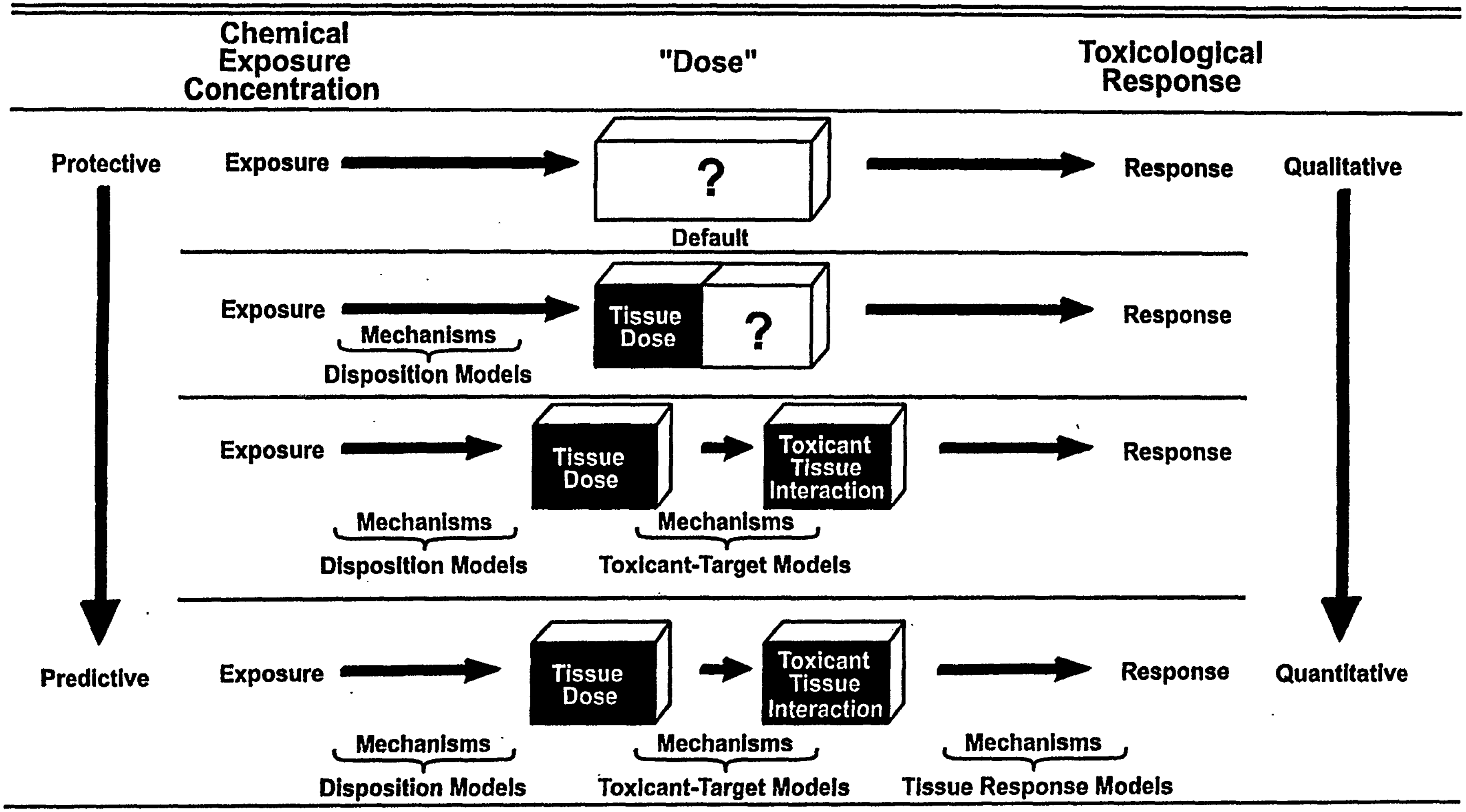
Rat to Human

Variability Across Humans

Schematic of Interspecies and Intrahuman UF Components Proposed for Perchlorate



Schematic Characterization of Comprehensive Exposure-Dose-Response Continuum and the Evolution of Protective to Predictive Dose-Response Estimates.



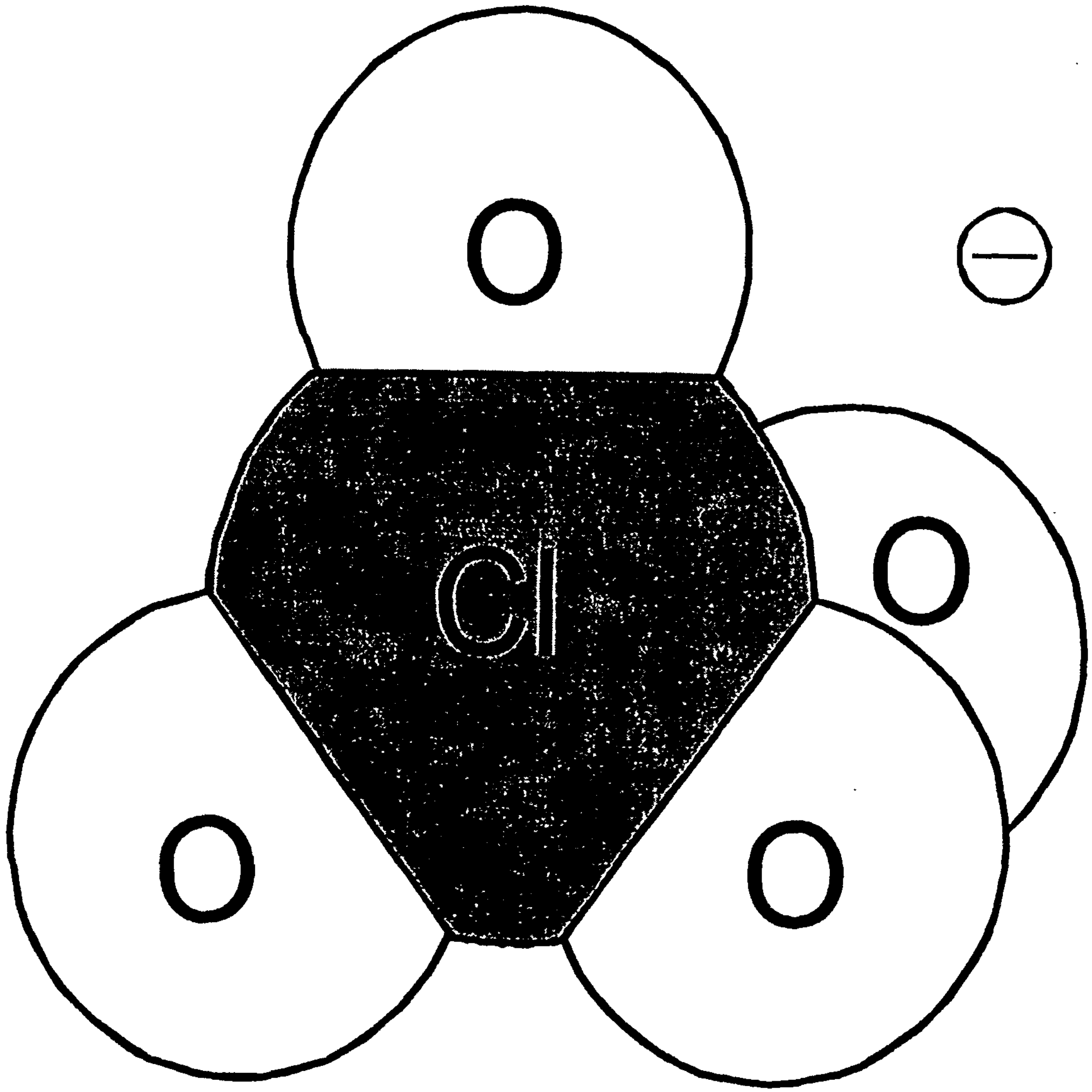
Provisional RfD Estimates

- **Principal study = Stanbury & Wyngaarden (1952)**
- **NOAEL = 0.14 mg/kg-day for 100% iodide release**
- **UF = 1000 (1992):**
  - intrahuman variability (10), less than chronic data (10), database deficiencies (10)
- UF = 300 (1995):
  - intrahuman variability (10), less than chronic data (10), database deficiencies (3)
- **Provisional drinking water action levels:**
  - **3.5 - 18 ppb based on 70 kg / 2 L water**

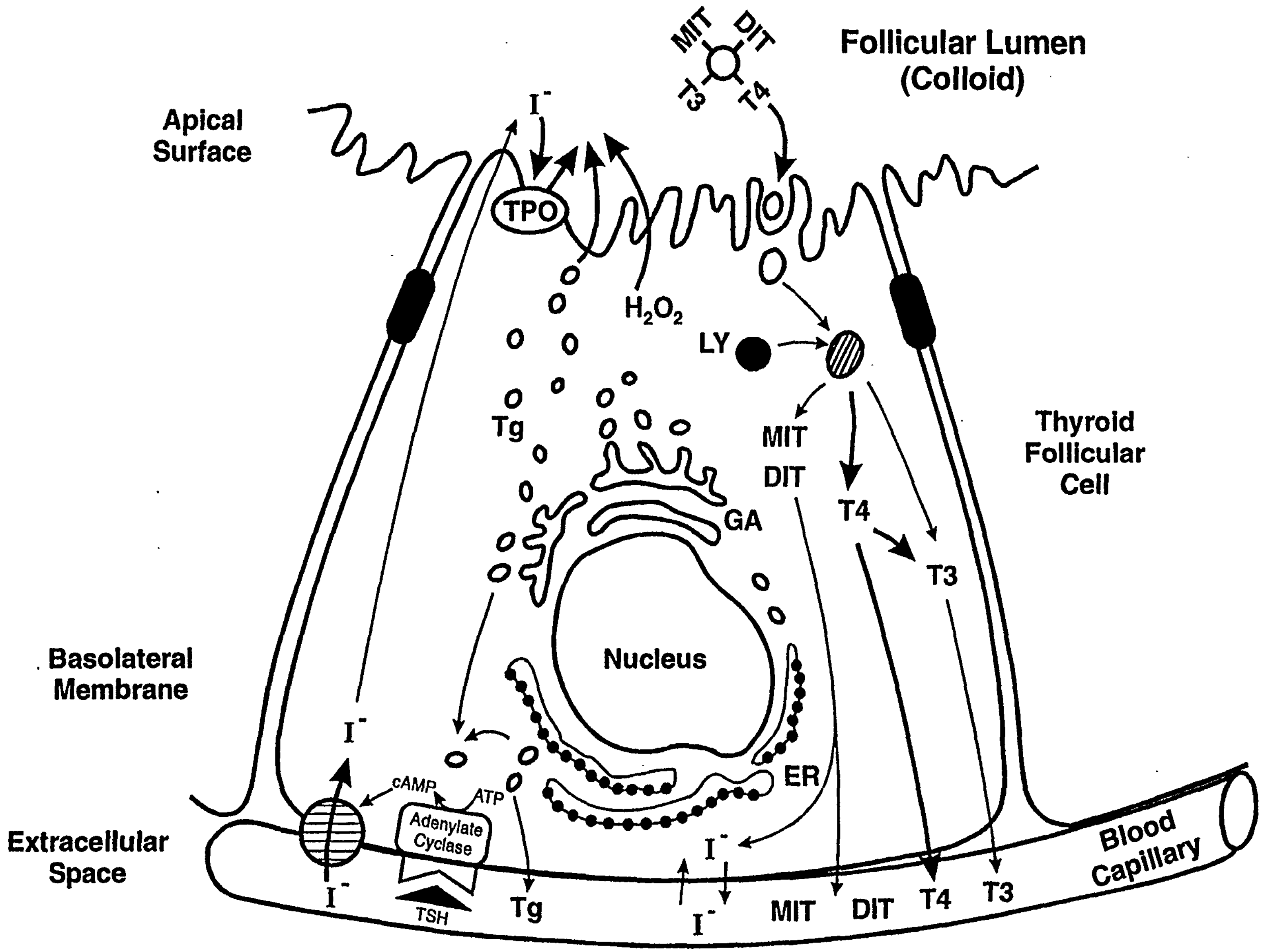
March 1997 TERA External Peer Review

- Same principal study, UF = 100:
  - intrahuman (3), subchronic to chronic(3), LOAEL to NOAEL (3), database deficiencies (3)
- Inadequate data base for RfD derivation
- Available mechanistic insights suggest special studies and synthesis strategy
- Eight (8) additional new categories of studies recommended

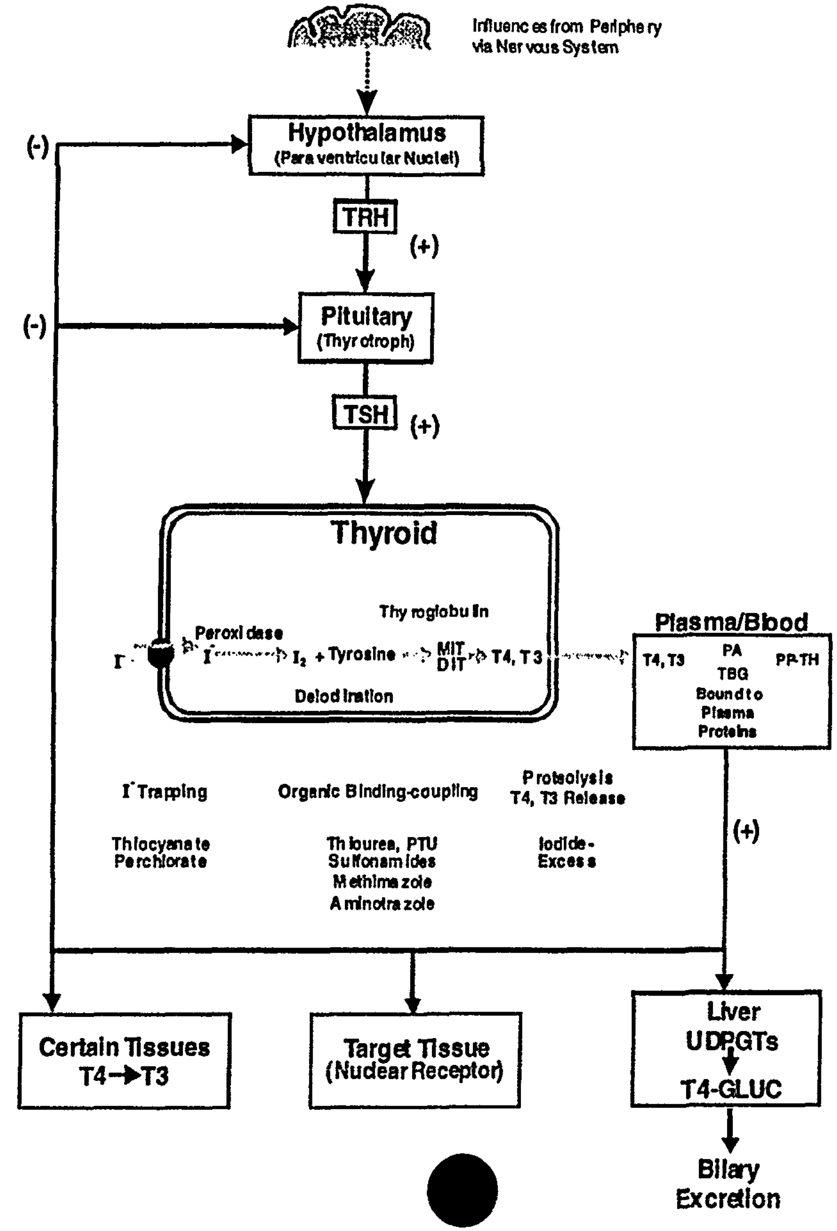
Chemical Structure of Perchlorate



Thyroid Hormone Biosynthesis and Secretion



Hypothalamic-Pituitary-Thyroid Axis and Feedback Mechanisms



INTERSPECIES AND INTRASPECIES DIFFERENCES IN THYROID STRUCTURE AND T3, T4, AND TSH HORMONES[a]

Parameter	Human	Rat
Thyroxine-binding globulin	Present	Essentially absent
T_4 Half-life	5 to 6 Days	0.5 to 1 Day
T_3 Half-life	1 Day	0.25 Day
T_4 Production rate/kg body weight	1 ×	10 × that in humans
TSH	1 ×	6 to 60 × that in humans
Follicular cell morphology	Low cuboidal	Cuboidal
Sex differences		
Serum TSH	M = F	M[d] ≤ 2 × F[e]
Cancer sensitivity	F = 2.5 × M	M > F

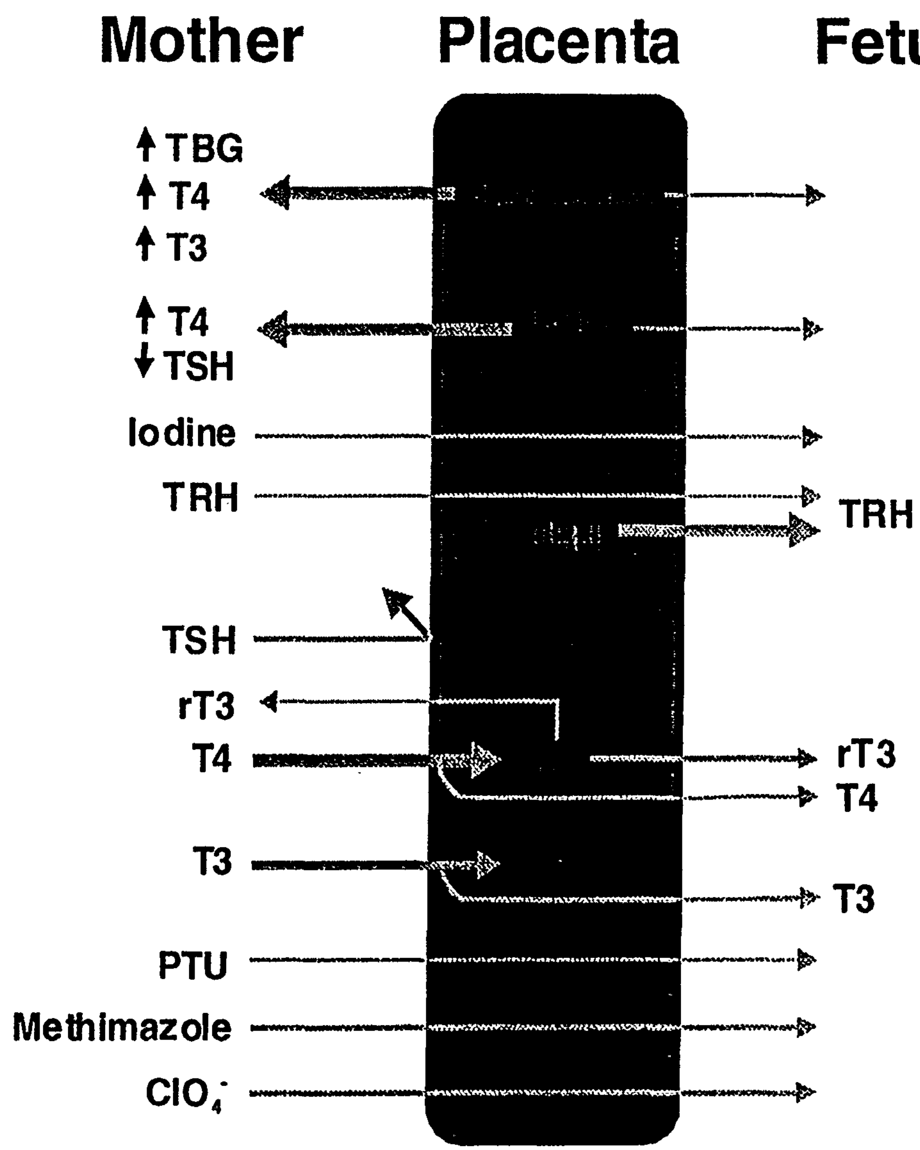
[a]M = male, F = female.

Source: U.S. Environmental Protection Agency (1998a).

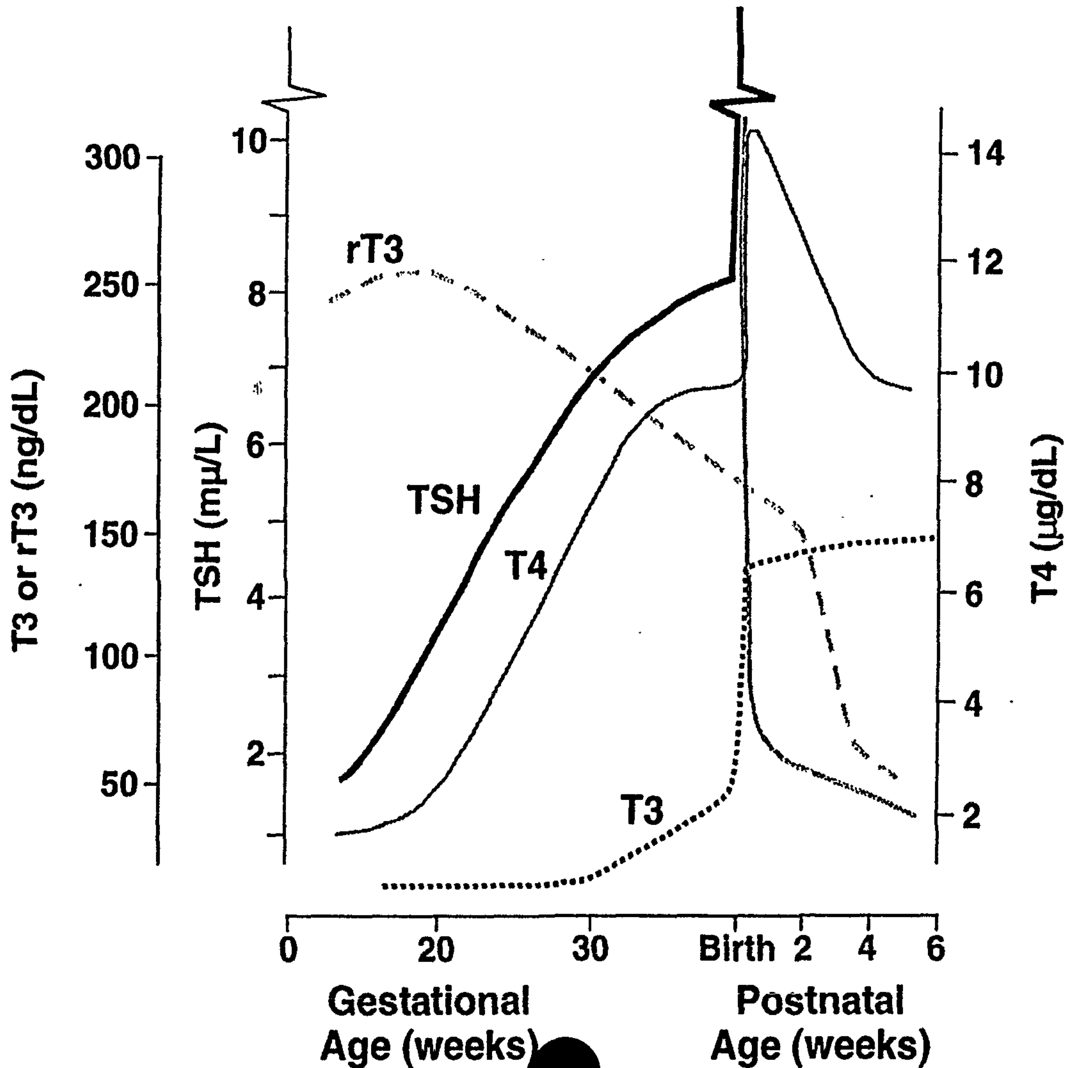
Main Symptoms and Effects of Hypothyroidism

Developmental *(Transient disruption leads to permanent effects.)*	Adult *(Transient disruption leads to transient effects.)*
• Delayed reflex ontogeny	• Run down, slow, depressed
• Impaired fine motor skills	• Sluggish, cold, tired
• Deaf-mutism, spasticity	• Dryness and brittleness of hair
• Gait disturbances	• Dry and itchy skin, constipation
• Mental retardation	• Muscle cramps
• Speech impairments	• Increased menstrual flow
	• Thyroid tumors in rodents

Role of Placenta in Human Thyroid Hormone Metabolism



Pattern of Change in Human Fetal and Neonatal Function Parameters During Pregnancy and Postnatal Periods



Mechanisms of Anti-Thyroid Mediated Neoplasia in Rodents

- DNA Directed:
  - X - rays
  - 131 I
  - Genotoxic chemicals
- Indirect
  - Partial thyroidectomy
  - Transplantation of TSH-secreting pituitary tumors
  - Iodide deficiency
  - Chemicals inhibiting iodide uptake
  - Chemicals inhibiting thyroid peroxidase
  - Chemicals inhibiting TH
  - Chemicals inhibiting conversion of T3 & T4
  - Chemical inhibiting hepatic thyroid hormone metabolism and excretion

Proliferative Lesions Thyroid Follicular Cells in Rodents

Morphologic Continuum

Normal

Hyperplasia

Adenoma

Carcinoma

Significance in Risk Assessment

Deficiencies of 1997 Data Base

- Human Clinical Studies
  - Adult subjects
  - Typically subjects with thyroids altered by disease or other treatments
  - Few pregnant subjects
  - Acute or short-term exposure duration
  - Limited range of doses
- Laboratory Animal Studies
  - Limited range of doses
  - Dated
- Additional Target Tissues Suggested
  - Reproductive function
  - Immunotoxicity (aplastic anemia, leukopenia)

Mode of Action Provides Important Insight to Characterization of Toxicity

- A chemical's influence on the molecular, cellular, and physiological functions in producing tumors
- Prolonged depression of TH causes a feedback that leads to upregulation of TSH which leads to thyroid gland hyperplasia
- Genotoxic?

Existing Data Summary

- Target tissue appears to be the thyroid but available testing not comprehensive across endpoints
- Anti-thyroid effects would differ among adult versus developing fetus, children
- Anti-thyroid effects associated with benign neoplasia development in rats; a nonlinear process
- Genotoxicity not characterized
- Relevancy to human risk of rat tumors not established; presumed protective

Recommended Studies

- 90-Day subchronic bioassay
- Developmental neurotoxicity study
- Genotoxicity assays
- Mechanistic studies
- ADME - Absorption, Distribution, Metabolism and Elimination
- Developmental study
- 2-Generation reproductive toxicity study
- Immunotoxicity

Developmental Study in Rabbits (Argus, 1998c)

- 0, 0.1, 1.0, 10, 30, and 100 mg/kg-day
- Developmental endpoints
  - Fetal NOAEL > 100 mg/kg-day
- Thyroid histopathology
  - Maternal NOAEL and LOAEL for hypertrophy at 1.0 and 10 mg/kg-day
- Maternal hormone analyses
  - EPA analysis designates LOAEL for T4 at 0.1 mg/kg-day
  - Lack of effect on T3 and TSH

2-Generation Reproductive Study (Argus, 1998b)

- 0, 0.3, 3.0 and 30 mg/kg-day (30/sex/group)
- Maternal organ weights
  - Thyroid increased at 3.0 and 30 in males and 30 mg/kg-day in females
  - Possibly also in pituitary
- Reproductive parameters
  - Hints of effects in the 0.3 mg/kg-day group in the mating, fertility, estrous cycle, ovarian weights

Analyses Submitted on 2/1/99 or 2/8/99

2-Generation Reproductive Study

- Thyroid weights and ratios
  - Stat sig in F1 pups males at 3.0 and 30 mg/kg-day; in females also at 0.3 mg/kg-day
- Histolopathology incidence and severity dose-related only in thyroid: hypertrophy, hyperplasia, decrease in colloid
  - P1 both sexes at all dose groups
  - F1 pups at 3.0 and 30 mg/kg-day
  - Require additional analyses

Preliminary Analyses

Analyses Submitted on 2/1/99 or 2/8/99

2-Generation Reproductive Study

- Hormone data for F0 and F1
  - No apparent trend
  - Require additional analyses
- Reproductive parameters (sperm morphology and estrous cyclicity)
  - Effects suggested in P1 not replicated in F1
- Final Audited Report due: 3/5/99

Immunotoxicity Studies at Medical University of South Carolina

(14-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"C, G, I, J, T, K"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured. Supplementary experiments were needed to acquire additional serum samples for hormone analysis or to repeat the NK assay.
"U, V"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and were challenged with listeria to assess **delayed type hypersensitivity.**
"H, F, M"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
SRBC Experiments	B6C3F1 female mice (two experiments of 30 mice each) were exposed to **90 days of AP** (0, 0.1, 3.0, or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 75, bled on day 79 to determine specific IgM antibody levels, and bled on day 90 to determine specific IgG antibody levels.

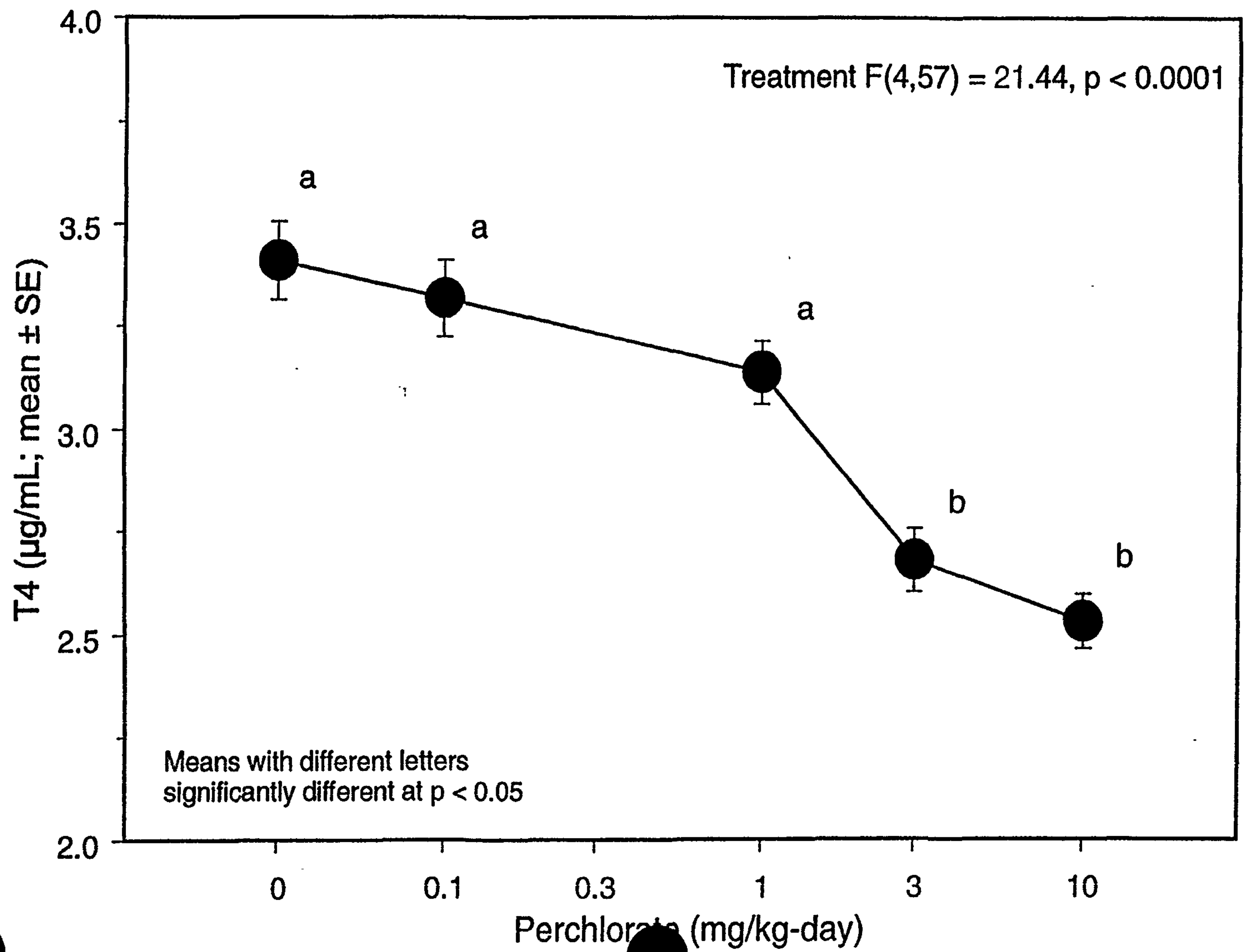
Immunotoxicity Studies at Medical University of South Carolina
(90-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"A, D, N"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of immune, hematological, or thyroid parameters were measured. In experiments "A" and "D", thyroid histopathology was performed. Experiment "N" included a variety of other parameters: macrophage phagocytosis and nitrite production, NKh, assay organ weights and cellularities, flow cytometry, and serum for hormone analysis.
"B, E"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured.
"P"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
"Q"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with **B16F10 melanomas** on day 76.
"L"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with ***Listeria monocytogenes*** on day 86.
SRBC Experiments	B6C3F1 Female mice (1 experiment of 30 mice) were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0 or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 9 and bled on day 14 to determine specific IgM antibody levels.

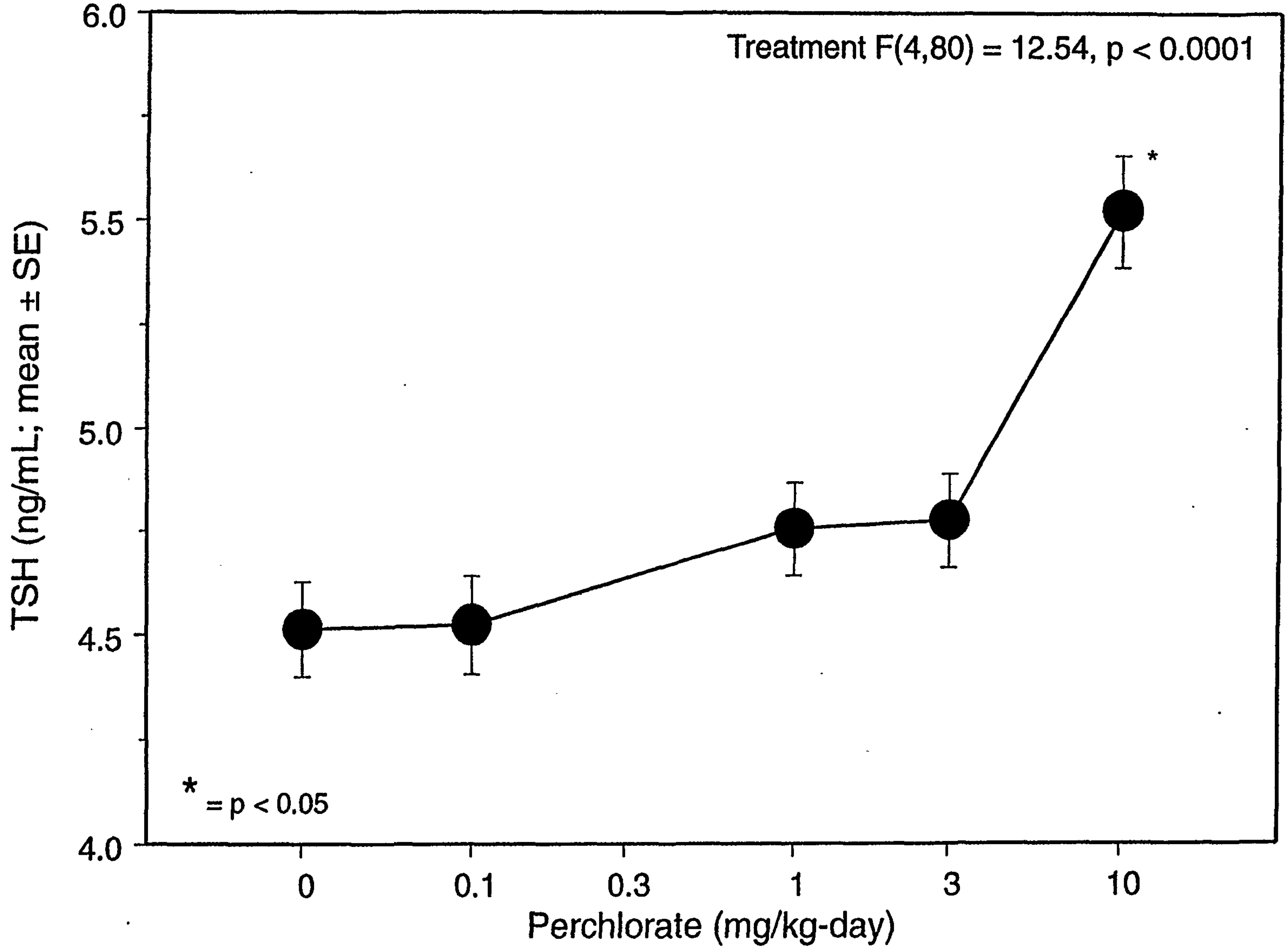
Analyses Submitted on 2/1/99 or 2/8/99
Immunotoxicity Studies

- Thyroid histopathology (Warren, 1999) in mice from 90-day (2 experiments) in immunotoxicity study @ MUSC
  - Control (0%) and 30 mg/kg-day high dose (100%); additional dose groups pending
  - Hypertrophy, hyperplasia, colloid depletion, congestion
  - Severity scores not provided
  - Likely useful to compare with 30 mg/kg-day dose of 2-generation reproductive
- 14-day and 90-day SRBC assays
  - Negative

Effects on Serum Total T4 in F1 Pups on PND5 (Data of Argus, 1998a)



Effects on Serum TSH Pups on PND5
(Data of Argus, 1998a)

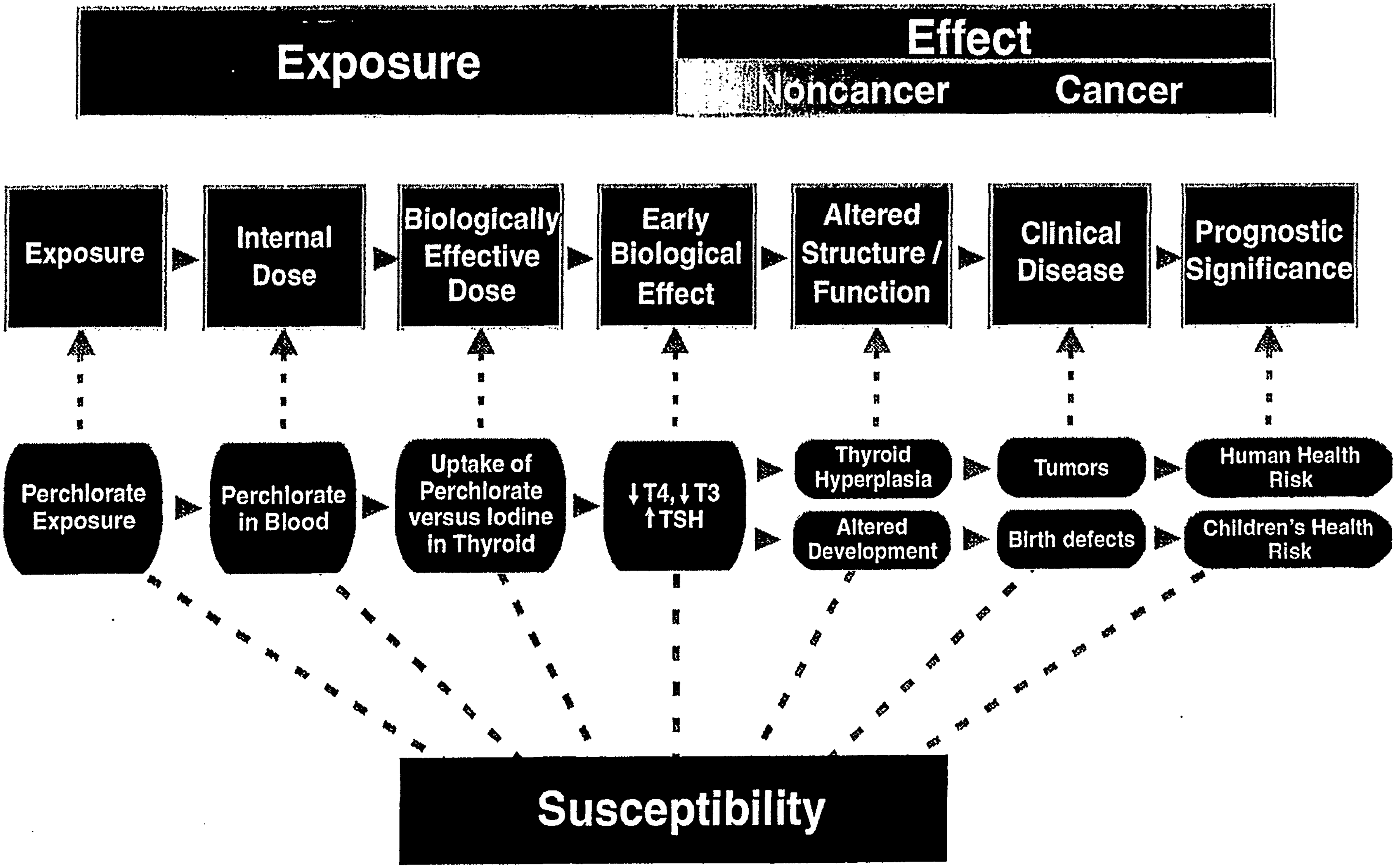


SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS

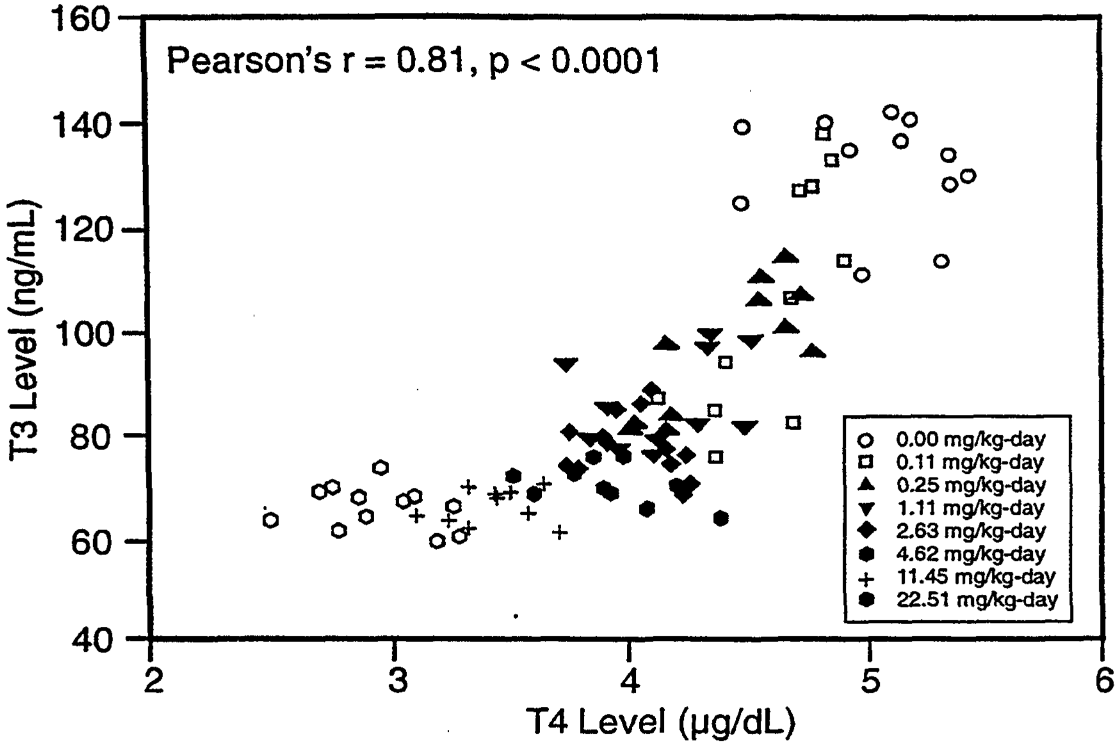
(Green cells designate NOAELs; Purple cells LOAELs; ✓ = dose tested)

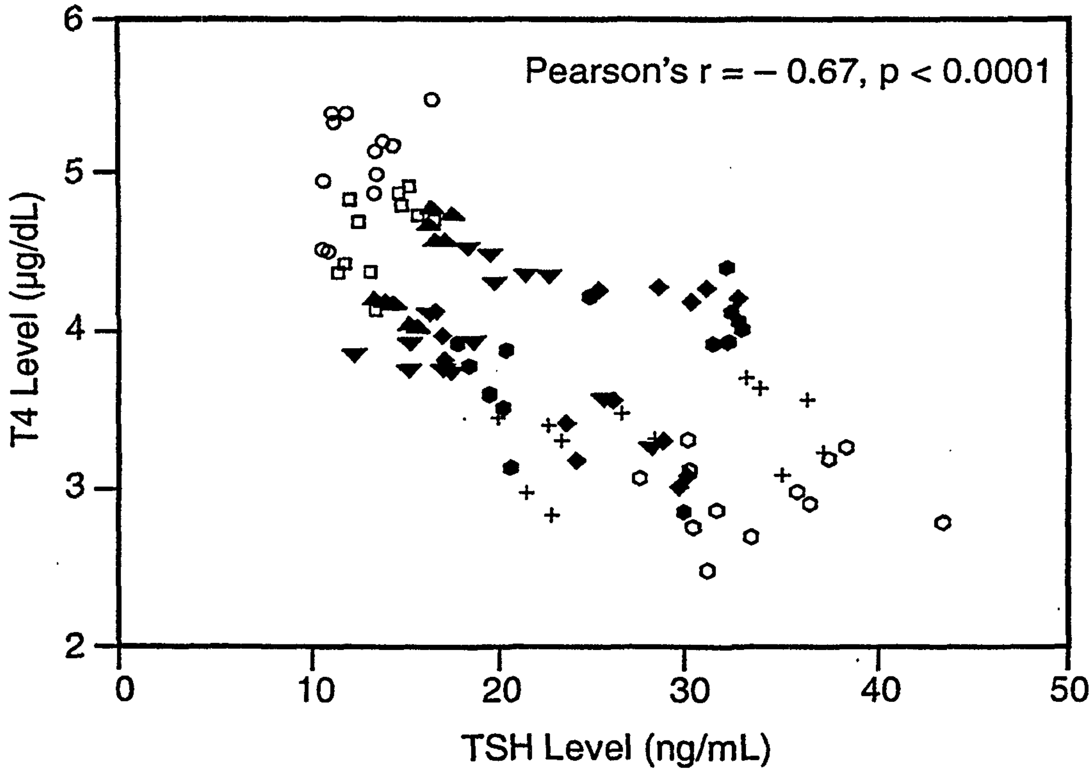
Study/Species	Dose Duration/ Age Tested	Sex	DOSES (mg/kg/day)												
			Effects	0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0
Caldwell 14-day Rat (Caldwell et al., 1995)	14 days	M&F	T3	✓			[illegible]	[illegible]	[illegible]	[illegible]	✓	[illegible]	✓	✓	✓
			rT3				M	✓	[illegible]	[illegible]	[illegible]	[illegible]	✓	✓	✓
			T4 hTG	✓			[illegible]	✓	[illegible]	✓	✓	[illegible]	[illegible]	✓	✓
			TSH	✓			F	✓	[illegible]	M	✓	✓	✓	[illegible]	✓
			Hist	✓			SH [illegible] SH FLS	[illegible]	[illegible]	SH [illegible] MH FLS	SH Thy [illegible] SH [illegible]	✓	✓	✓	✓
Subchronic Rat (Springborn Laboratories, Inc., 1998)	14 days	M&F	T3	✓	M	M	[illegible]	M	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	M	
			T4	✓	✓	✓		✓			✓			✓	
			TSH	✓	F	F M	[illegible]	✓	[illegible]	[illegible]	✓	[illegible]	[illegible]	✓	
			Hist	✓	✓	✓		✓			SH FCH Thy-wt			✓	
	90 days		T3	✓	[illegible]	✓	[illegible]	✓	[illegible]	[illegible]	✓	[illegible]	[illegible]	✓	
			T4	✓	✓	✓	[illegible]	✓	[illegible]	[illegible]	✓	[illegible]	[illegible]	[illegible]	
			TSH	✓	✓	✓		✓	[illegible]	[illegible]	✓	[illegible]	[illegible]	✓	
			Hist	✓	✓	✓		✓			SH FCH Thy-wt			✓	
	120 days		T3	✓							✓			✓	
			T4	✓		✓	[illegible]	[illegible]	[illegible]	[illegible]	✓	[illegible]	[illegible]	✓	
			TSH	✓		✓					✓			✓	
			Hist	✓		✓					✓			✓	

Proposed Mode-of-Action Model for Risk Assessment of Perchlorate

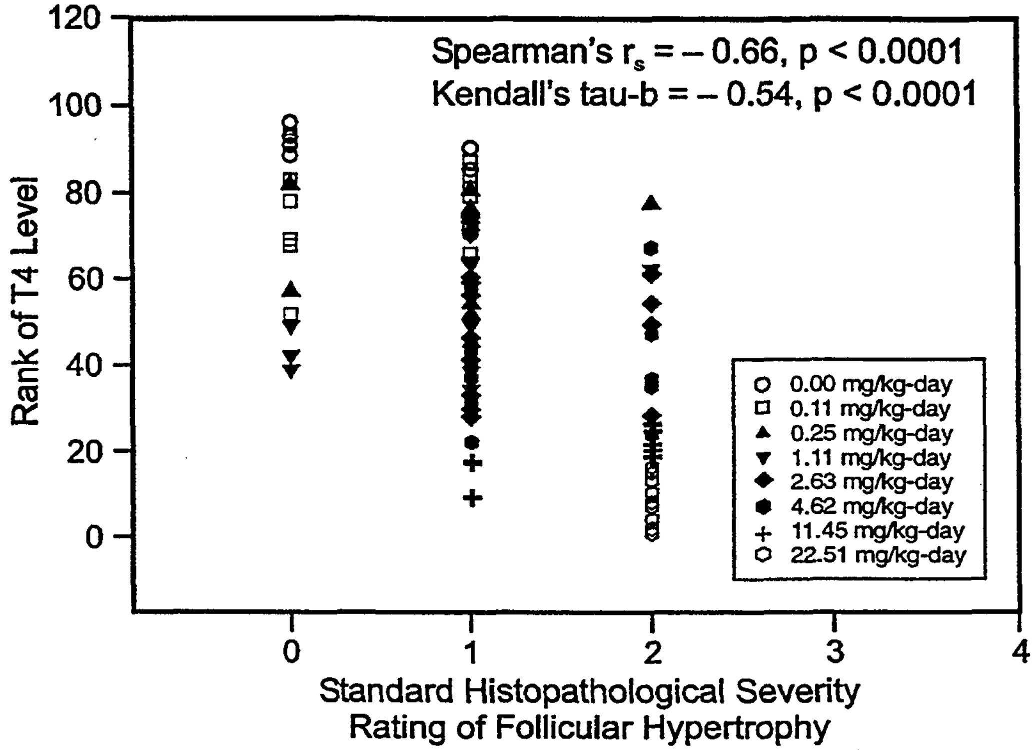


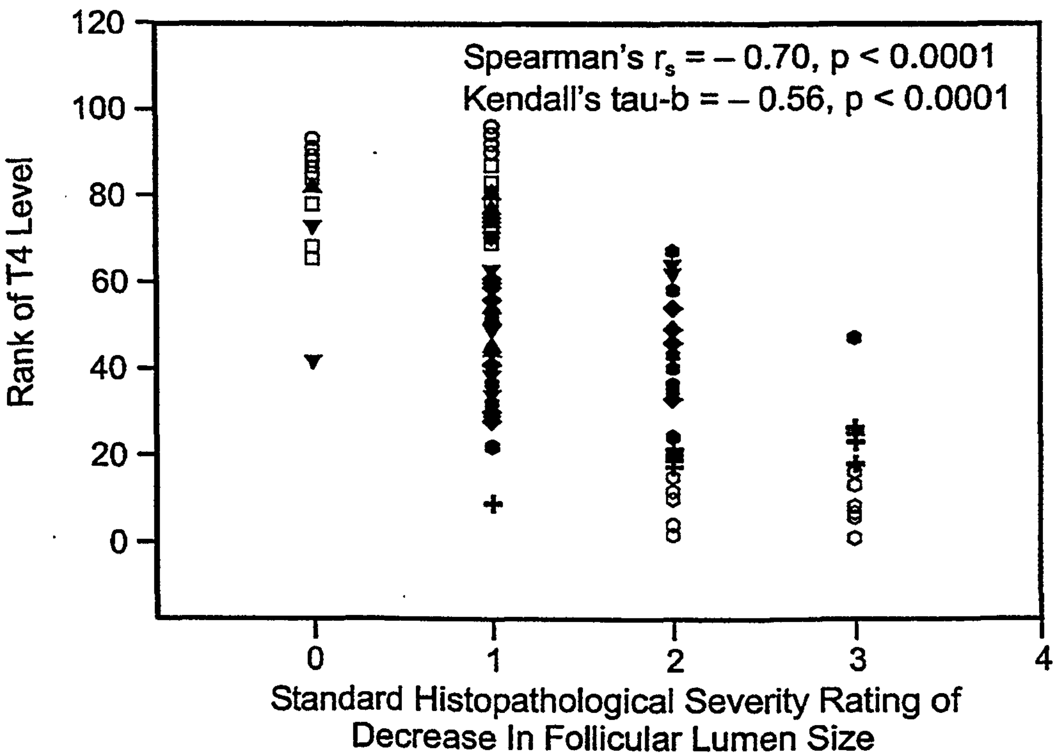
CORRELATIONS BETWEEN T3 AND T4 OR T4 AND TSH IN RATS FROM 14-DAY STUDY (CALDWELL ET AL., 1995)



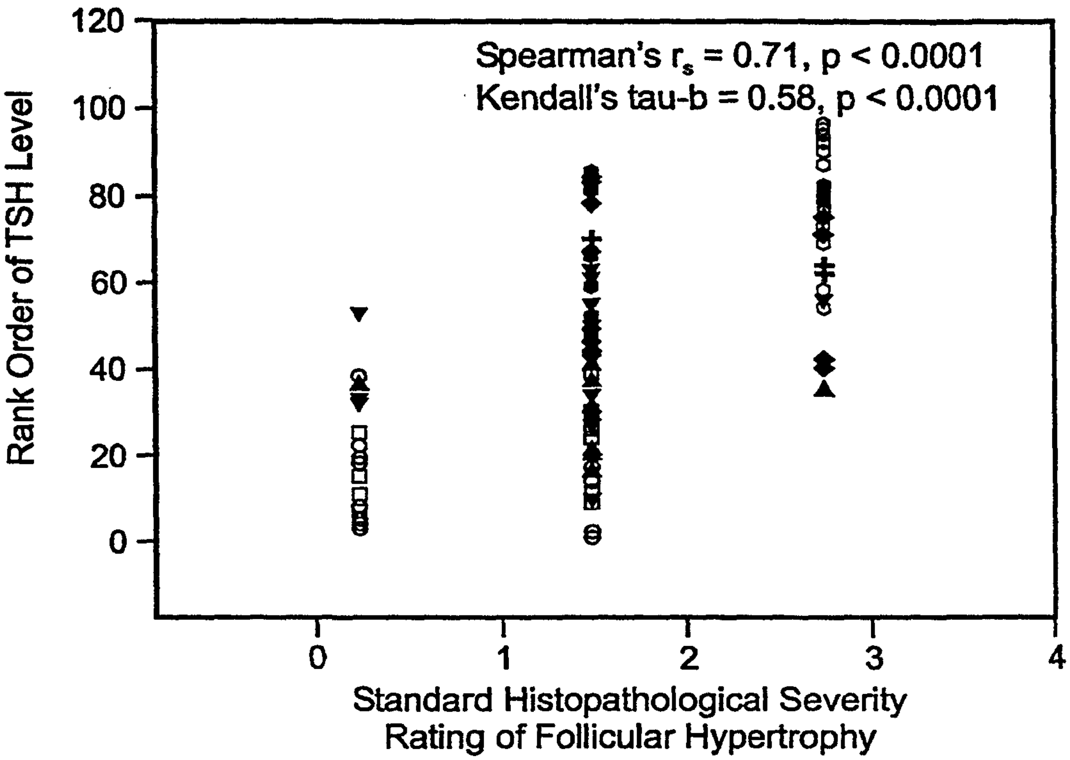


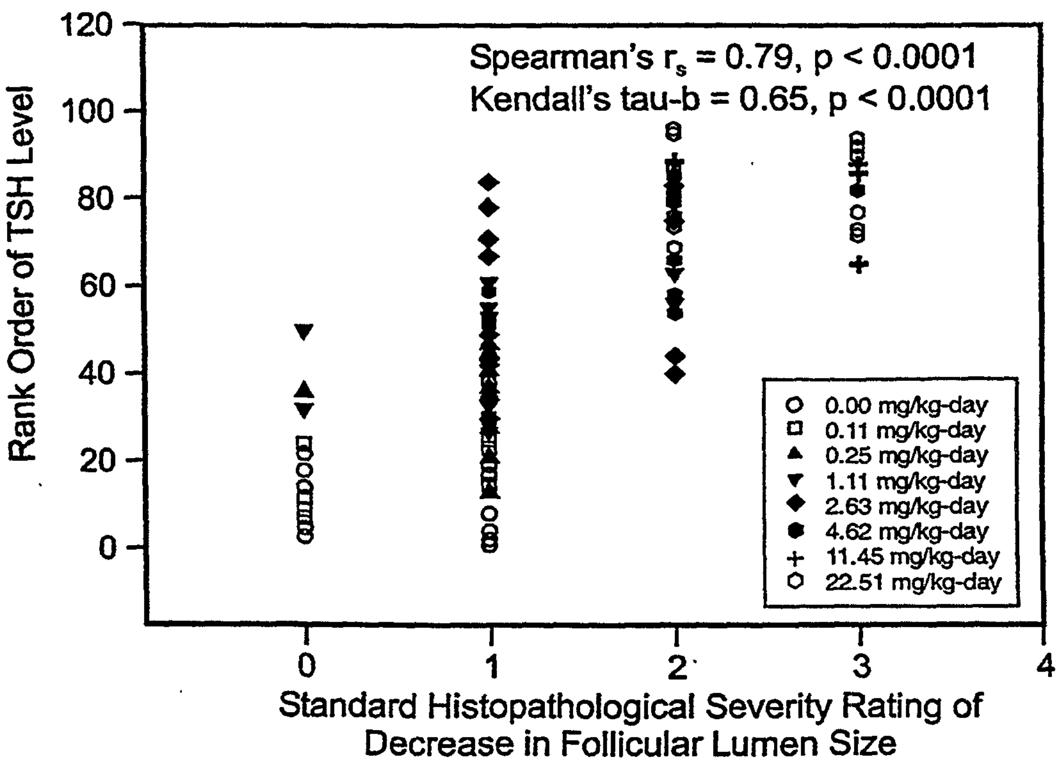
CORRELATIONS BETWEEN RANK ORDER OF 54 AND STANDARD HISTOPATHOLOGY SEVERITY RATING FOR LESIONS IN THYROIDS OF RATS FROM 14-DAY STUDY (CALDWELL ET AL., 1995)





CORRELATIONS BETWEEN RANK ORDER OF TSH AND STANDARD HISTOPATHOLOGY SEVERITY RATINGS FOR LESIONS IN THYROIDS OF RATS FROM 14-DAY STUDY (CALDWELL ET AL., 1995)





SUMMARY OF HORMONE (T3, T4, and TSH) AND HISTOLOGY EFFECTS

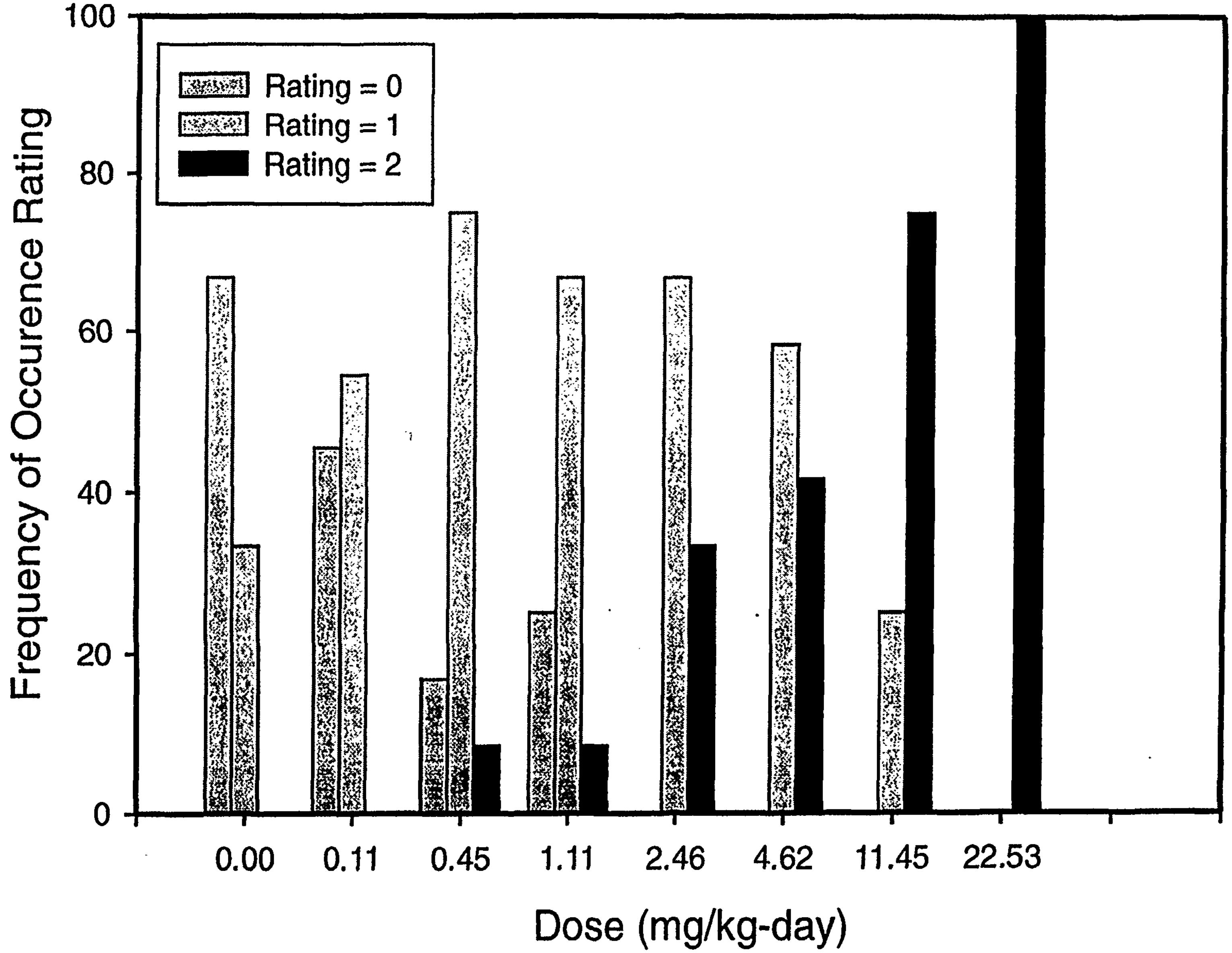
(Green cells designate NOAELs; Purple cells LOAELs; ✓ = dose tested)

Study/Species	Dose Duration/ Age Tested	Sex	DOSES (mg/kg/day)												
			Effects	0	0.01	0.05	0.1	0.2	0.3	0.44	1.0	3.0	5.0	10.0	30.0
Developmental Neurotoxicity Rat (Argus Research Laboratories, Inc., 1998a)	FO: PP10	F	T3	✓											
			T4	✓											
			TSH	✓											
			Hist	✓			✓				SH [illegible]				
	F1: PND5	M&F	T3	✓			✓								
			T4	✓			✓				✓				
			TSH	✓			✓				✓	✓			
			Hist	✓											
			Hist				✓				MH				
	F1: PND90	M&F	T3	ND							ND	ND		ND	
			T4	ND							ND	ND		ND	
			TSH	ND							ND	ND		ND	
			Hist[4]	✓							✓	20%		80%	

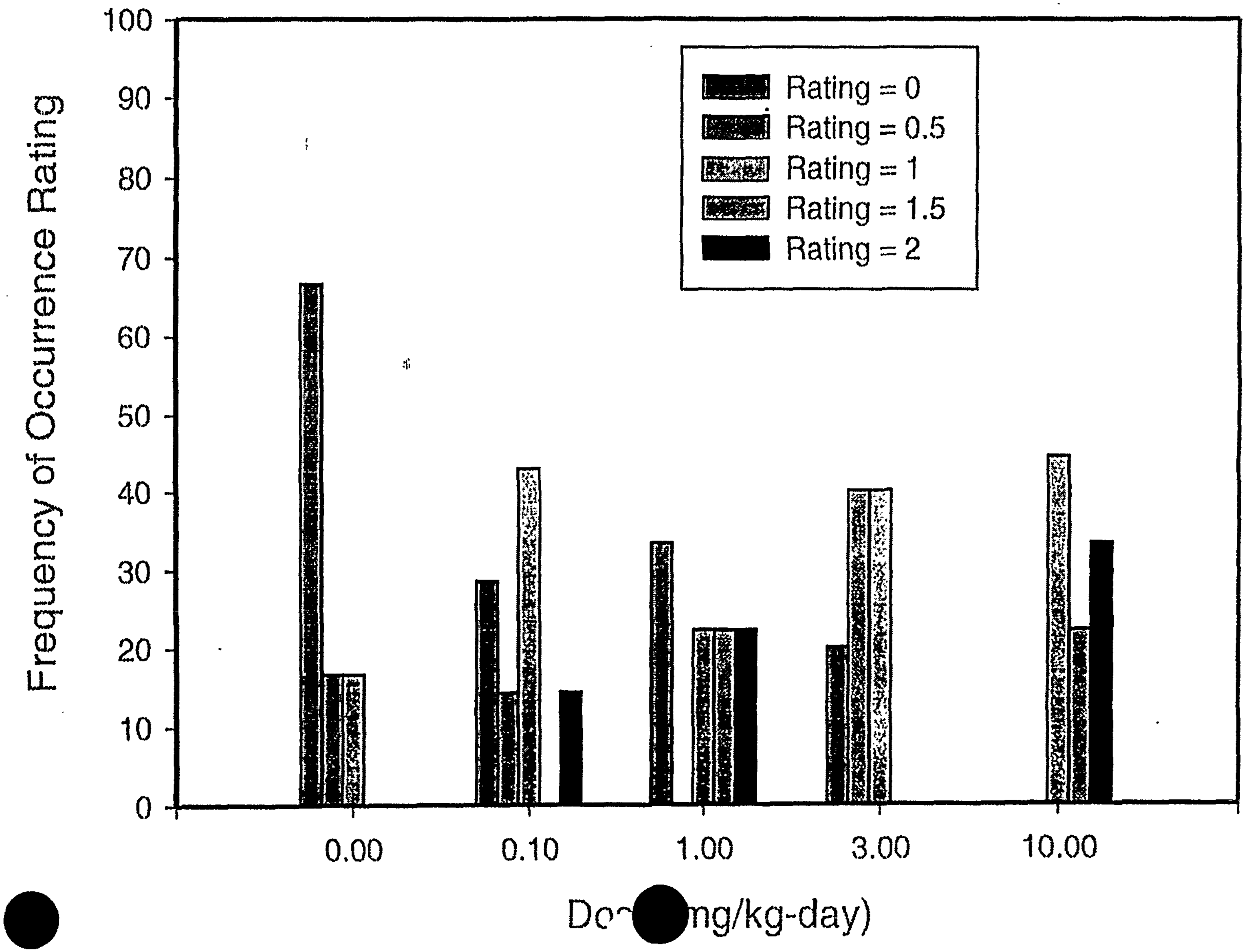
COMBINED INCIDENCE DATA AND AVERAGE SEVERITY SCORES FOR MALE AND FEMALE PND5 RAT PUPS FOR FOLLICULAR EPITHELIAL CELL HYPERTROPHY AND DECREASE IN FOLLICULAR LUMEN SIZE BASED ON STANDARD HISTOLOGY

		Perchlorate (mg/kg-day)				
Measure	Present	Control	0.1	1.0	3.0	10.0
Cell hypertrophy	Incidence[a]	3/12	8/12	9/12	8/12	12/12
	Severity[b]	0.33	0.84	1.08	0.83	1.42
Lumen Size	Incidence	6/12	10/12	10/12	11/12	12/12
	Severity	0.66	1.17	1.25	1.75	2.16

Frequency of Occurrence by Dose Group of Each Standard Histopathological Severity Rating for Follicular Epithelial Cell Hypertrophy in Rats of 14-Day Caldwell et al. (1995) Study



Frequency of Occurrence Per Litter By Dose Group of Each Standard Histopathological Severity Rating for Follicular Cell Hypertrophy in F1 Rat Pups on PND5 (Argus, 1998a; York, 1998a, b, c, d, e)

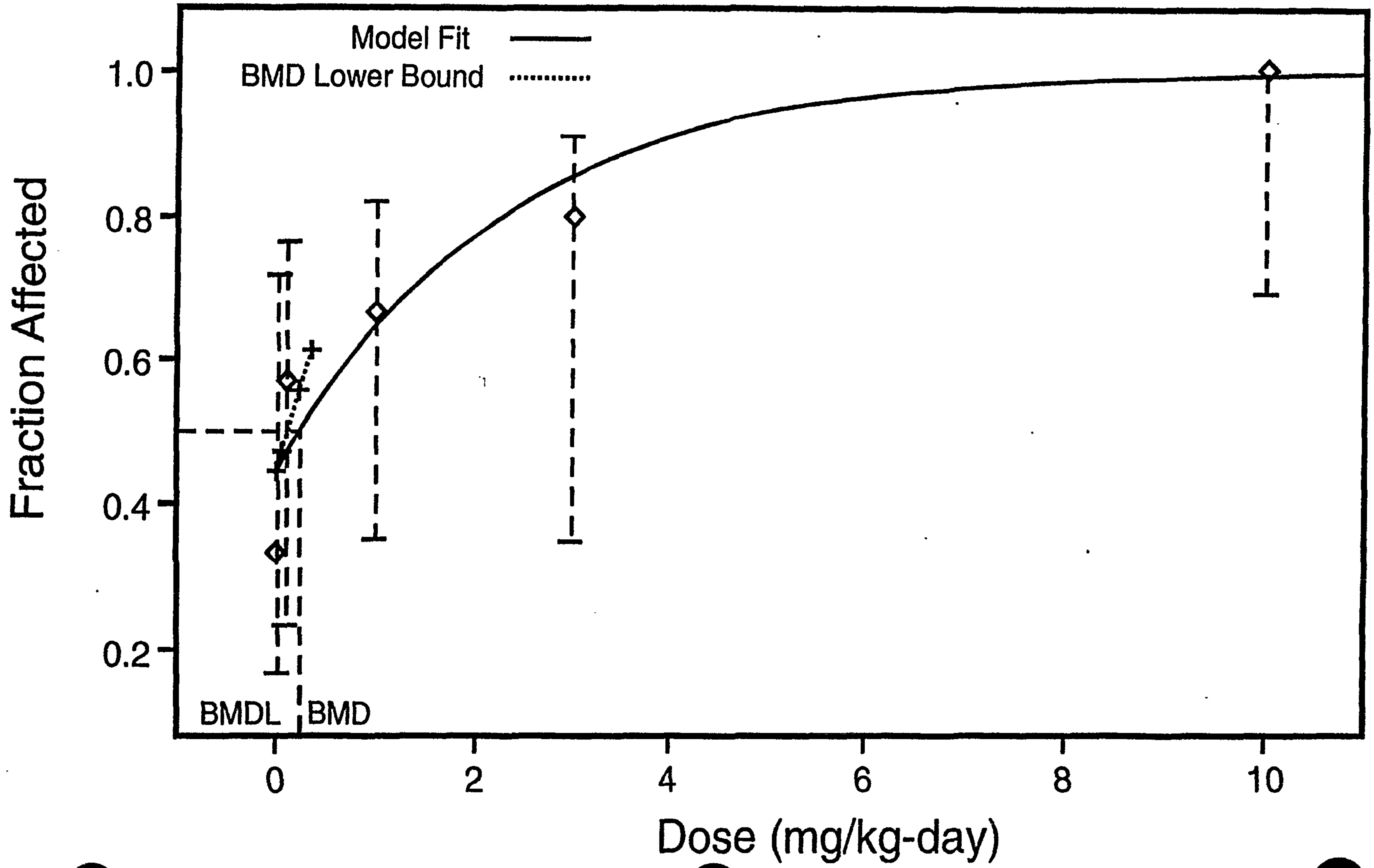


Benchmark Dose (BMD) and BMD 95% Lower Limit (BMDL) Estimates of the Incidence of Follicular Epithelial Cell Hypertrophy in the F1 Pups on PND5 From the Developmental Neurotoxicity Study (Argus, 1998a)

(Benchmark response based on 10% extra risk.)

Model	p of fit,df	BMD	BMDL	LOAEL	BMD: LOAEL	BMDL: LOAEL
Gamma	0.85, 3	0.234	0.10	0.1	2.34	1.0
Logistic	0.84, 3	0.35	0.27	0.1	3.5	2.7
Probit	0.84, 3	0.379	0.376	0.1	3.79	3.76
Quantal Linear	0.85, 3	0.234	0.10	0.1	2.34	1.0
Quantal Quadratic	0.74, 3	0.96	0.53	0.1	9.6	5.3
Weibull	0.85, 3	0.234	0.10	0.1	2.34	1.0

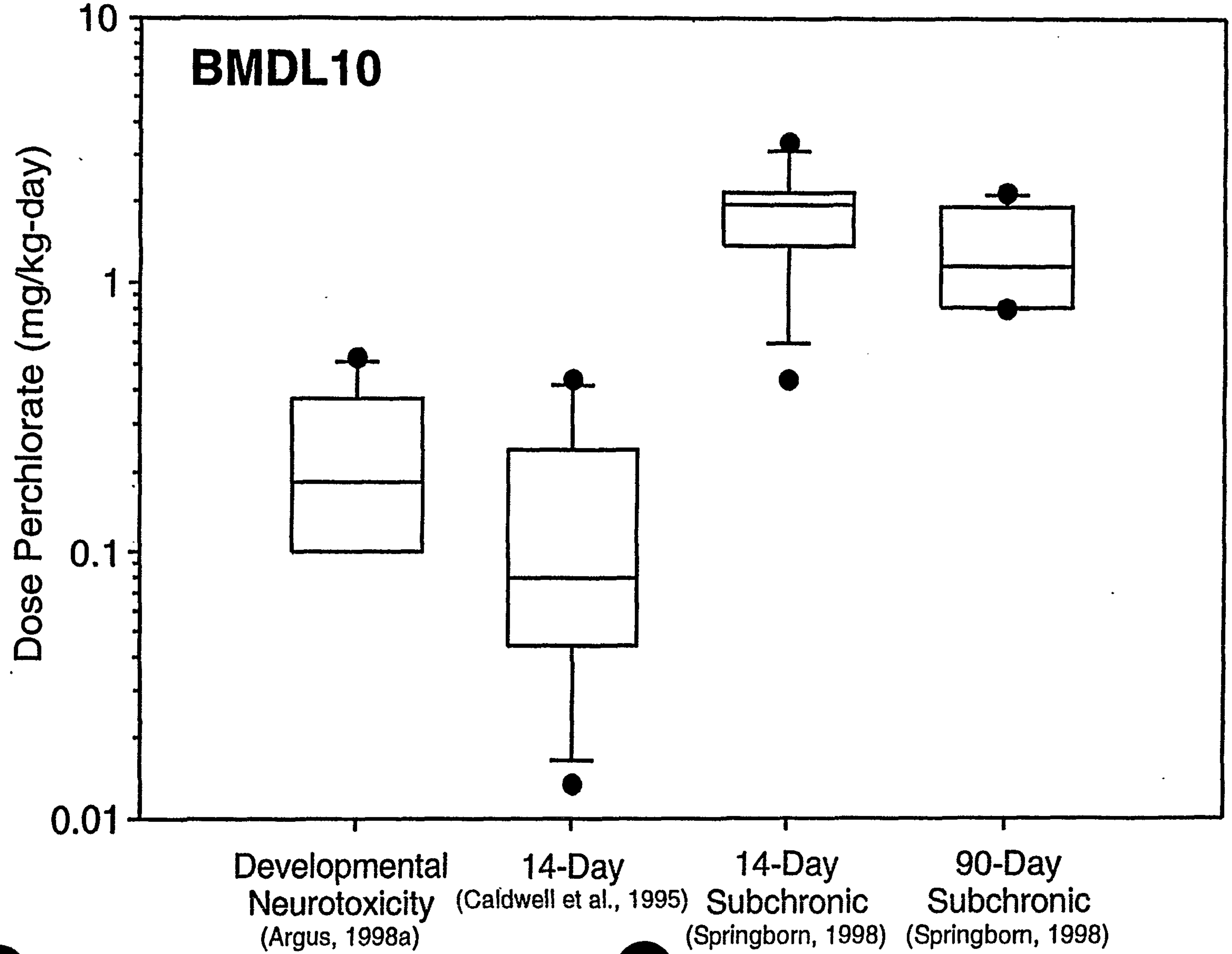
Model Fit to the Litter-by-Litter Incidence of Follicular Cell Hypertrophy (Standard Histopathology) in F1 Rat Pups on PND 5 (Argus, 1998a; York a, b, c, d, e)



Analyses Submitted on 2/1/99 or 2/8/99
Neurodevelopmental Study

- Brain histopathology at the 3 mg/kg-day dose
  - Decrease in size of hippocampal gyrus (-12%) and caudate putamen (-7%); but no difference between control and high
  - Pending commentary on U-shaped awaits PBPK
- Nonparametric reanalysis of thyroid histopathology in pups on PND5
  - Exact tests reinforce concern for effect at 0.1 mg/kg-day; especially with small sample
- Litter-by-litter BMD analysis
  - BMD and BMDL virtually identical to Geller, 1998b

Lower Limit on 10% Response Benchmark Dose (BMDL) of Standard Histopathology in Rat Thyroids



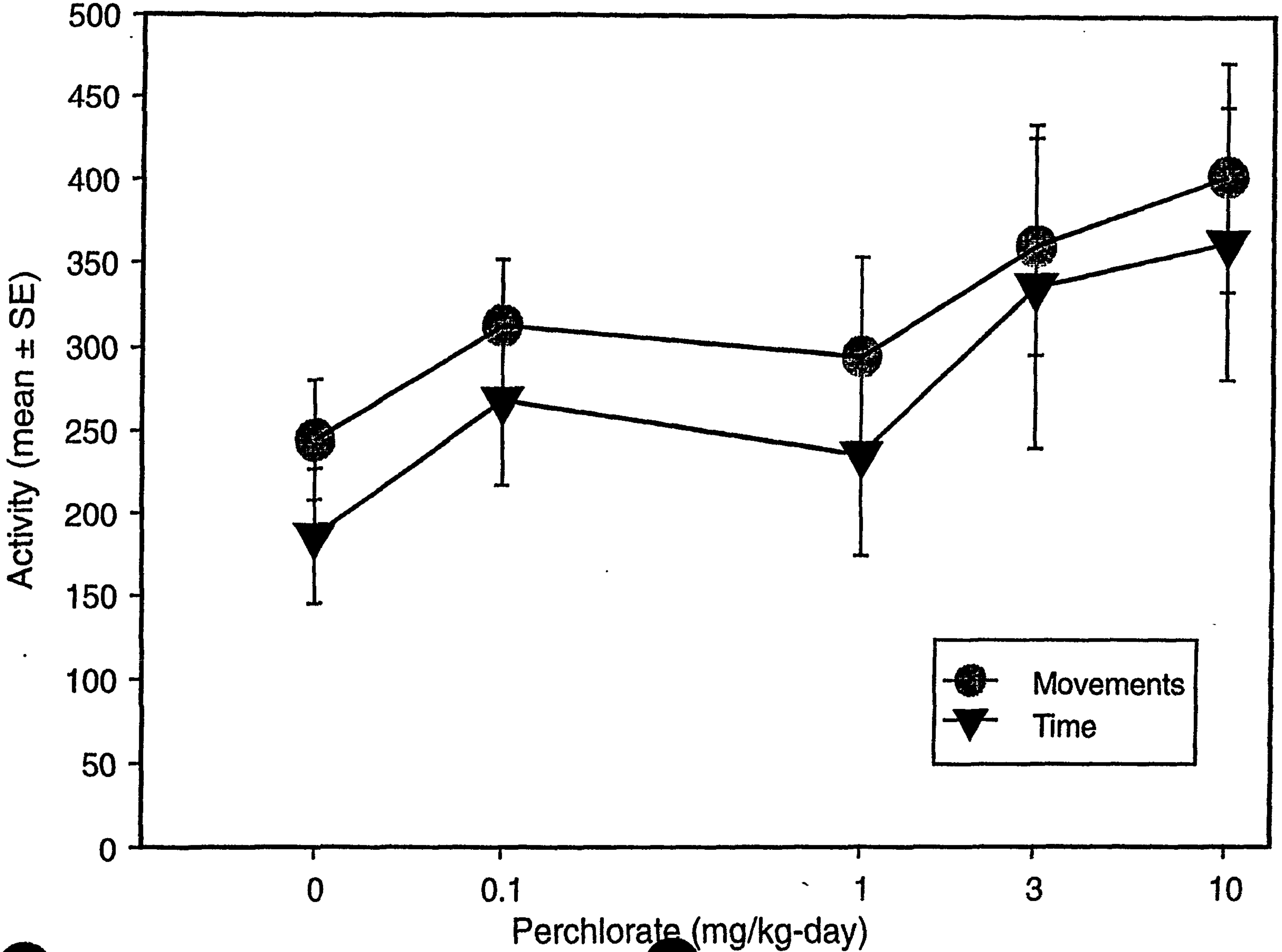
BENCHMARK DOSE (BMD) AND BMD 95% LOWER LIMIT (BMDL) ESTIMATES FOR COMBINED MALE AND FEMALE HORMONE DATA OF 14-DAY TIME POINT IN THE SPRINGBORN (1998) SUBCHRONIC STUDY

(Benchmark response based on 10, 20, and 40% changes from control value.)

Endpoint	Model	p of Fit	BMD BMDL (10%)	BMD BMDL (20%)	BMD BMDL (40%)	Mean	NOAEL
T4	Power	0.203	1.16 0.0035	12.73 1.21	138.94 38.33	5.066	1.0
ln(T4)	Power	0.22	0.037	3.899	36.48		1.0
T3	Power	0.41	0.000033 —	0.207 —	129.39 0.129[a]	166.5	0.01[b]
ln(T3)	Power	0.35	Lower limit includes 0	0.000054[a]	43.16[a]		0.01[b]
TSH	Power	0.45	0.037 0.000076	0.326 0.005	2.89 0.36	12.616	0.01
ln(TSH)	Power	0.43	0.0015	0.098	6.587		0.01

[a]BMDL calculation failed at a number of values. This means BMDL value may not be accurate.
[b]LOAEL, not NOAEL.

Perchlorate Effects on Motor Activity in Male Rat Pups on PND 14 (Argus, 1998a)



Lower Limit on Benchmark Dose for Motor Activity

(Response Levels at Control + 10, 20, 30 or 40% of Control)

	p of Fit	+10% of Ctl	+20% of Ctl	+40% of Ctl	Ctl mean (std dev)	Estimated mean
Movement	0.72	1.04	2.08	4.17	244.5 (162.75)	273.04
Time	0.69	0.66	1.33	2.67	186.05 (184.78)	239.07

Interpretation Issues
Histopathology

- Standard histopathology versus morphometry on lumen size
- Four different pathologists across various studies
- Different severity rating scales
- No formal QA by second pathologist
- Pending Proposal: PWG of all histopathology

Interpretation Issues
Hormone Analyses

- Three different laboratories
- Considerable variability

- Pending study: Interlaboratory validation study

Pending Data

Pilot Study for Inter-Laboratory Variability of Hormone Analyses

- For T3, T4, TSH:
  - Obtain 3 standards (high, med, low) and three rat samples
  - Send to three labs as unknowns
  - Each lab runs standards using SOP & normal source for RIA kit / chemicals
  - AFRL/HEST will run the 6 samples using our source for kits plus source of kits / chemicals for the other two labs
  - All data will be sent to EPA or 3rd party for statistical analysis

Data Analyses

- EPA conducted extensive reanalysis of submitted data
  - Some too simplistic (e.g., lack of gender in the model
  - Some data were not analyzed (e.g., incidence data
  - A lot of data was subjected to benchmark analyses
- Future Needs
  - Some analyses and graphics need corrections per reviewer comments
  - New analyses on studies in progress

Evidence for Indirect Carcinogenic Antithyroid Mode of Action

- Demonstrated dose-dependent effect in both fetal and adult stages in thyroid follicular cell hypertrophy / hyperplasia, lumen size and colloid reduction
- Hormone (T3, T4, TSH) changes correlated with histopathology acorss a number of studies and time points
- Site of action clearly at symporter, possibly not only locus

Evidence for Indirect Carcinogenic Antithyroid Mode of Action

- Reversibility demonstrated in thyroid weight, hypertrophy, hyperplasia and hormones after 30-day recovery of 90-day studies in rats and mice; possibly in pups on PND10 and PND22
- Progression of lesions between 14-day and 90-day time points
- Genotoxicity battery negative

Completed Analyses

Genotoxicity Battery

- NTP repeat (Zeiger, 1999*a*) of mouse micronuclei assay by ip injection found perchlorate was not toxic or mutagenic at 125, 250 or 500 mg/kg-day. All animals died in the 1500 and 2000 mg/kg-day groups; 4/5 at 1000.
- Repeat of mouse lymphoma by BioReliance negative.
- EPA conclusion: Perchlorate is not mutagenic.

Completed Analyses

Genotoxicity Battery

- NTP repeat (Zeiger, 1999b) of Salmonella (Ames test) mutagenicity battery found perchlorate negative at doses of 100 to 10,000 μg / plate.
- 10% and 30% S-9 concentrations used from Aroclor induced hamster (HLI) and rats (RLI)
- Tester strains TA102, TA104, TA100, TA1535, TA97 and TA98

"RfD" Derivation

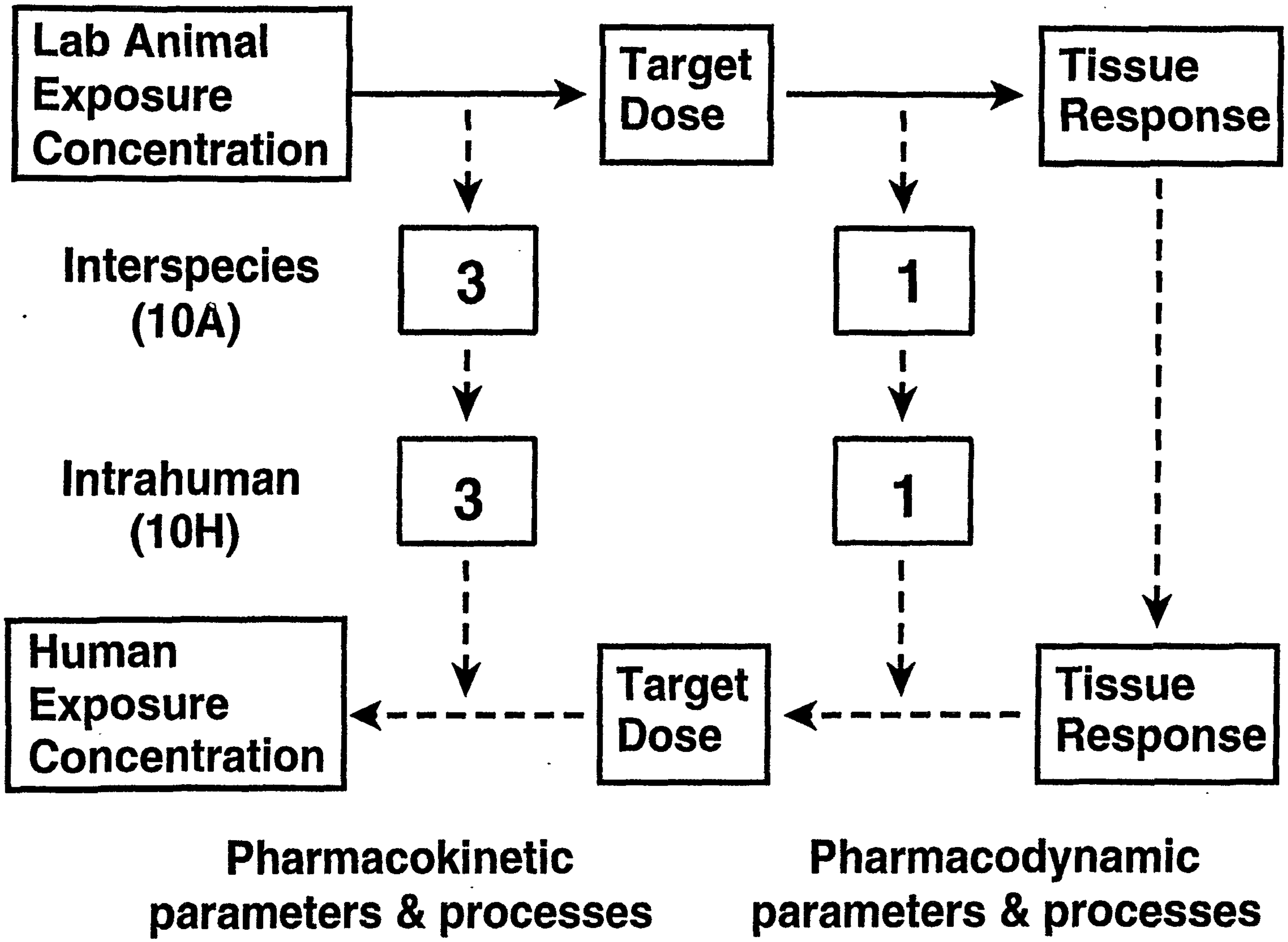
- LOAEL = 0.1 mg/kg-day
- Adjustment for molecular weight of ammonium perchlorate = 0.85
- Composite UF = 100
- "RfD" = 0.0009 mg/kg-day
- Confidence in study, database, and "RfD" is medium

Uncertainty Factors

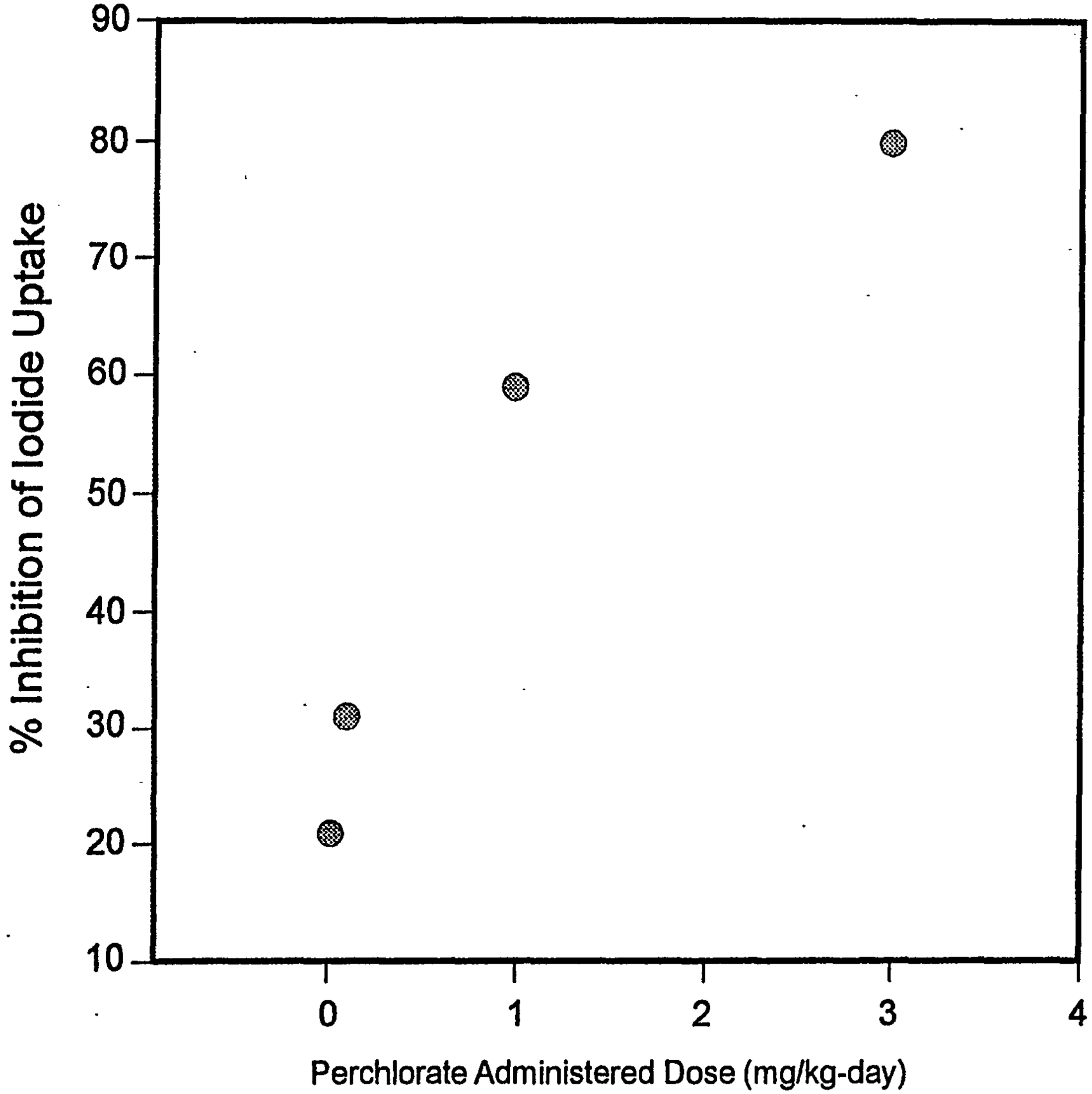
- Intrahuman: 3 for PK, PD partially covered by LOAEL to NOAEL factor
- Interspecies: 3 for PK
- LOAEL to NOAEL: 3 for minimal histopathology and concern over hormone data interpretation
- Data base deficiencies: 3 for lack of 2-generation reproductive and immunotoxity

- TOTAL COMPOSITE UF: 100

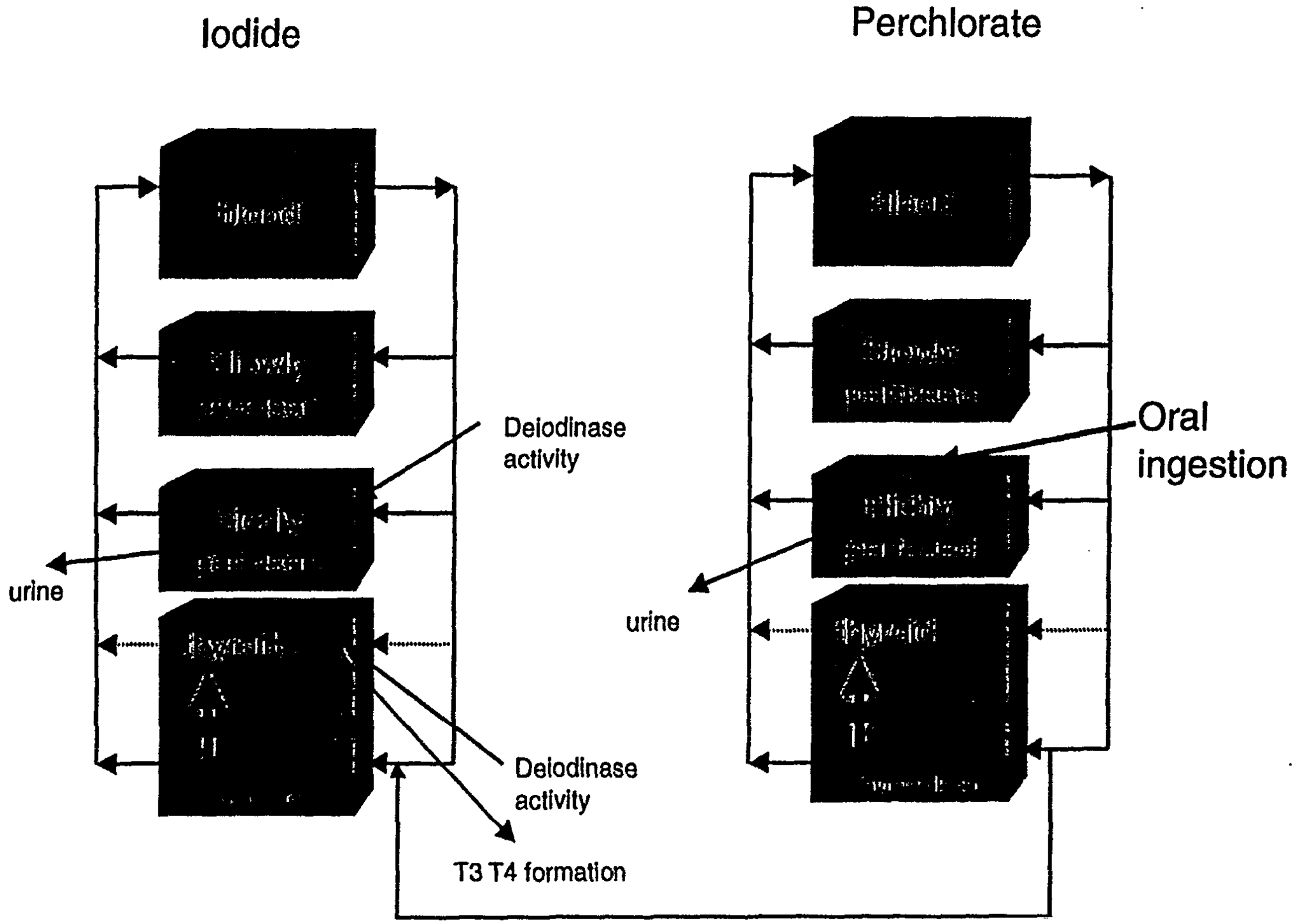
Schematic of Interspecies and Intrahuman UF Components Proposed for Perchlorate



Inhibition of Iodide Uptake by Oral Perchlorate Administration (Single 1p Dose)



Schematic of PBPK Model That is Under Development



Preliminary Analyses

Internal Dose Metric Correlations Using Data Submitted 2/5/99

- Meyer (1998) iodide uptake inhibition 9 hr after single iv dose
- Channel (1999) measured iodide uptake inhibition in rats dosed for 14 days by drinking water

Preliminary Analyses

Internal Dose Metric Correlations Using Data Submitted 2/5/99

- Caldwell 14-day study
  - Only 4 of 9 doses
  - Iodide uptake inhibition correlated better with T4 and T3 than did administered dose for single dose
  - TSH approximately same correlation for single dose
  - Worse with 14-day data than with administered
- Neurodevelopmental study
  - Only 4 of 5 doses
  - Correlations with administered or iodide inhibition significant and approximately similar for single dose
  - Correlations were worse with iodide inhibition than for administered dose for 14-day data

Preliminary Analyses

Internal Dose Metric Correlations Using Data Submitted 2/5/99

- Subchronic study
  - 3 of 6 doses tested
  - 14-day timepoint
    - Correlations for T3 and TSH marginally better with iodide uptake inhibition for single dose; T4 not
    - Correlations were worse with 14-day iodide uptake than for administered dose
  - 90-day timepoint
    - Correlations marginally higher with iodide uptake for single dose
    - Correlations were worse with 14-day iodide uptake than for administered dose

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Address dosimetry in dams, fetuses, and pups; repeat motor activity measures
- Timeline
  - Protocol development: 1 Mar 99
    - Submission to EPA as Consultative Letter
  - Protocol approval: 1 Apr 99
  - Begin studies: 1 May 99

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Pregnant Females / Fetuses
- Start dosing 2 wk prior to pregnancy
- Prenatal
  - Time points at GD 15 and 20
  - Determine inhibition of iodide uptake in the fetuses and dams
  - Measure T3,T4, TSH and perchlorate in blood
  - Thyroid histopathology

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Lactating Females / Pups
- Start dosing 2 wk prior to pregnancy
- Postnatal
  - Time points at PND 5, 10 and weening (PND 22)
  - Determine inhibition of iodide uptake in the pups and dams
  - Measure T3,T4, TSH and perchlorate in blood
  - Thyroid histopathology

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Pregnant Females / Fetuses
- Single dose
- Prenatal
  - Time points at GD 20
  - Determine inhibition of iodide uptake in the fetuses and dams
  - Measure T3,T4, TSH and perchlorate in blood

Pending Data

KINETIC STUDIES OF PREGNANT RATS, FETUSES AND PUPS

- Lactating Females / Pups
- Single dose
- Postnatal
  - Time point at PND 5
  - Determine inhibition of iodide uptake in the pups and dams
  - Measure T3,T4, TSH and perchlorate in blood

Pending Data

MOTOR ACTIVITY OF PUPS IN KINETIC STUDY

- Lactating Females/Pups
- Start dosing 2 wk prior to pregnancy
- Postnatal
  - Time point at PND 14
  - Conduct motor activity measurements to repeat data in Neurobehavioral Developmental Study
  - Pups then can be used for kinetic study time point at weening

Pending Data

HUMAN STUDY
Dr. Brabant (Medizinsche Hochschule) in Collaboration with AFRL/HEST

- Timeline
  - Conduct study: Feb and Mar 99
  - Data Analysis: Mar and Apr 99
  - Report Submission: May 99

Pending Data

HUMAN STUDY
Dr. Brabant (Medizinische Hochschule) in Collaboration with AFRL/HEST

- 14 day toxicity / kinetic study
- 3 doses, 7 male subjects per dose
- Each subject as own control
- Parameters to be measured
  - cytopuncture for PCR and iodide
  - body weight and thyroid ultrasound
  - CBC, T3, T4, TSH, Tg
  - iodide, perchlorate in blood and urine

Pending Data

HUMAN STUDY
Dr. Brabant (Medizinsche Hochschule) in Collaboration with AFRL/HEST

- Blood draws
  - prior to second dose on day one
  - daily on days 2-14 and days 1-3 post dosing
- Urine collection
  - 24 hour collection on days 1, 7, 14
  - 24 hour collection on days 1-3 post dosing

Pending Data

HUMAN STUDY
Dr. Braverman (Harvard Med School)
Funded by PSG

- 14 day toxicity study
- 1 dose level, 10 male subjects per dose
- Each subject as own control
- Parameters to be measured:
  - iodide^{123} in thyroid
  - body weight and physical exam
  - CBC, chem profile, urinalysis
  - TSH, T4 (serum and free), T3, Tg, TPO
  - iodide, perchlorate in blood and urine

Pending Data

HUMAN STUDY
Dr. Braverman (Harvard Med School)
Funded by PSG

- Blood draws
  - baseline
  - days 7, 14
  - amendment requested for day 15 at 2, 4, 8, 24 hrs after last dose
- Urine collection
  - 24 hour collection prior to days 1, 7, 14
  - 24 hour collection on days 1-5 post dosing (voluntary now - amendment requested)
- Amendment requested for 2 more doses

Preliminary Analyses

Analyses Submitted on 2/8/99

Human Studies

- Ecological Epidmiology Study (Lamm and Doemland, in press)
  - No evidence for increased incidence of congenital hypothyroidism in county and ethnic-specific data on newborns in NV and CA in 1996-1997
  - Limitations with respect to exposure estimates

Preliminary Analyses

Analyses Submitted on 2/8/99

Human Studies

- Cross-sectional Occupational Study of Workers Exposed via Inhalation (Lamm et al., in press)
- Ammonium Perchlorate Production workers (n = 37) in 3 categories (low, medium, high); sodium azide workers as controls (n = 21)
- Medical questionnaire, clinical chemistry, thyroid function, urine excretion pre- and post-shift for perchlorate, iodine, creatinine; CBC, Hg, HCT, MCV, MCH, MCHC

Preliminary Analyses

Analyses Submitted on 2/8/99

Human Studies

- Measures of respirable and total particles were highly correlated. MMAD for high exposure group (n =15) of 7.4 um with a geometric standard deviation of 1.8
- Absorption demonstrated by urinary excretion
- No evidence for thyroid or effects on other parameters
- Quantitative route-to-route extrapolation may be possible using these data

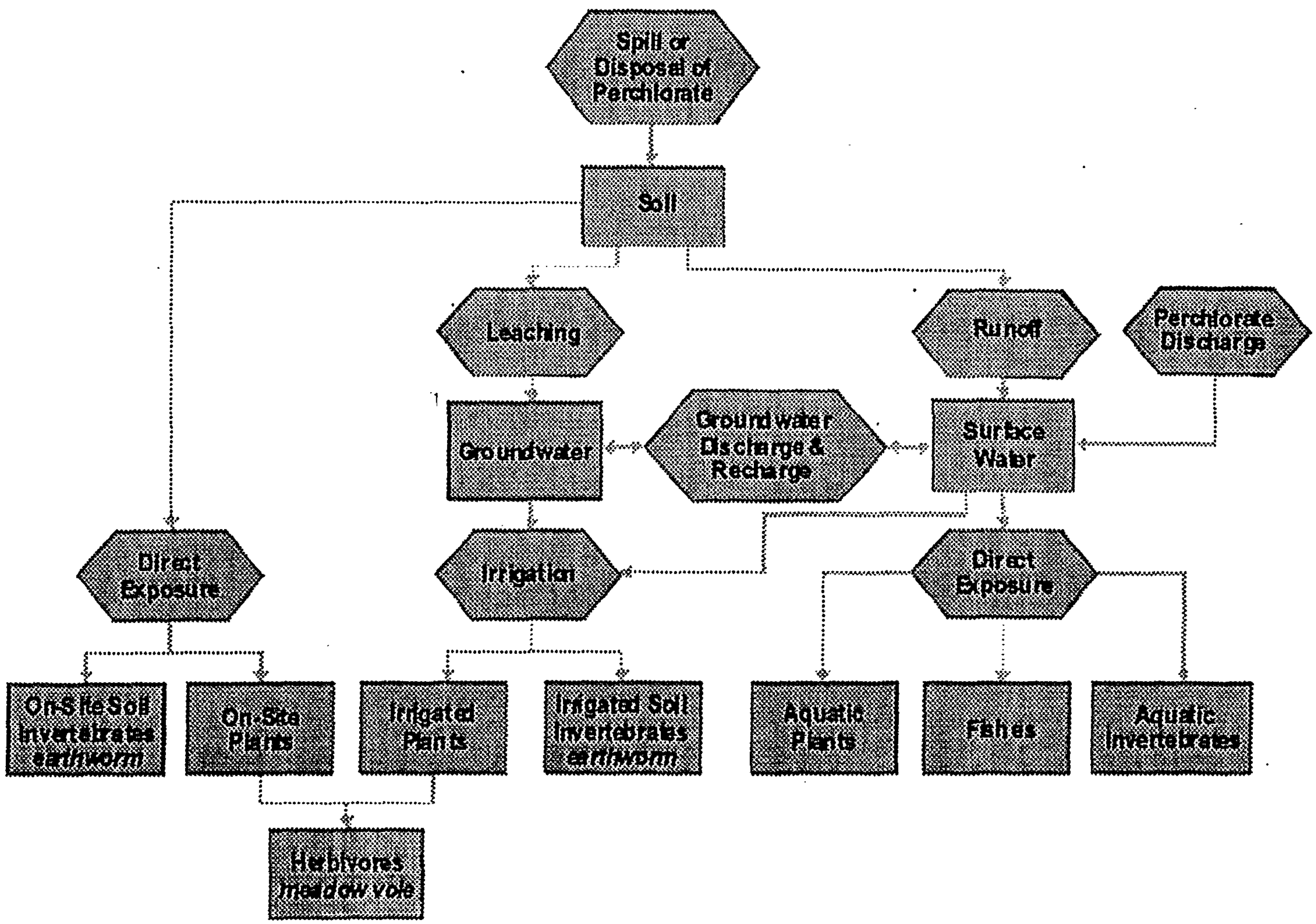
Ecological Screening Assessment

- Limited in scope due to database
  - IPSC report including literature search and screening battery
  - LC50 aquatic chronic toxicity testing
  - Frog Embryo Teratogenesis Assay: Xenopus (FETAX)
  - Phytotransformation study
- Scope responsive to stakeholder concerns

Ecological Screening Assessment

- Problem formulation focused on selection of assessment endpoints
  - Fish community richness and productivity
  - Aquatic invertebrate community richness and productivity
  - Aquatic plant productivity
  - Soil invertebrate community richness and productivity
  - Terrestrial plant productivity
  - Population productivity of herbivorous wildlife

Conceptual Model of Exposure of Ecological Receptors to Perchlorate



Ecological Screening Assessment

- Secondary acute value of 5 mg/L (as perchlorate) to be protective of aquatic organisms with 90%
- Quartile inhibitory concentrations for terrestrial plant growth in soil and sand at 78 mg/kg (293 mg/L) and 41 mg/kg (160 mg/L). A factor of 10 applied for interspecies variance to arrive at screening benchmark of 4 mg/kg.
- Limited data on invertebrates; conservative estimate at 1 mg/kg.
- A factor of 10 applied to human health risk LOAEL to obtain screening benchmark of 0.01 mg/kg-day for herbivores

Ecological Screening Assessment
Uncertainties and Research Needs

- Accurate exposure information
- Accurate linkage between biologically effective dose and degree of perturbations in hormones and neurobehavioral
- Data on bioaccumulation
  - Aquatic biota
  - Terrestrial vascular plants
- Effects on nondaphnid invertebrates and dietary exposure of birs and herbivorous or litter-feeding invertebrates
- Fate and transport in irrigated soils

Ecological Studies Under Consideration

- Screening animal exposure studies
  - Texas Tech (funded 99-00)
- Additional plant exposure studies are planned under the direction and management of Brooks Air Force Base
- Further plant studies are under consideration to be jointly funded between the USAF and EPA

Additional Proposed Studies

- Development of Analytical Methodologies for Plant Tissues
- Analytical Support and Pathway Analysis
- Plant Tissue Cultures to Define Plant Effects
- Mass Balances to Define Reaction Pathways and Kinetics
- Identification of Enzyme Systems
- ELISA to Identify Perchlorate Adaptable Plants
- Plant Selection and Evaluation
- Soil Chemistry
  - Sorption
  - Competitive Uptake
  - Growth Impacts
- Pilot Treatability Investigation
- Field-Scale Water Balance
- Plume Fate and Transport
- Development of Transgenic Plants
- Evaluation of Perchlorate Content in North American Fertilizers

Further Needs

- Evaluate how the proposed studies will further the recommended research needs
- Prioritize the studies for funding
- Establish appropriate management documentation
- Obtain funding
- Start studies

Conclusions

- Hormone and histology resutls support proposed mode of action model
- Uncertainties to be better addressed by pending studies
- Require recommendations on path forward to final document

Appendix J

Additional Analysis from EPA's National Center for Environmental Assessment

Table 1. Data Analyses Provided in February 1, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Final genetox assays a) Repeat of Salmonella battery plus 2 additional strains by NTP b) Repeat of mouse micronuclei assay by NTP c) Repeat of mouse lymphoma by BioReliance	Final — Memos and revised text to document provided.	David Brusick
2. Brain histopathology at the 3 mg/kg-day dose from the Argus (1998a) neurodevelopmental study	Preliminary pending recommendations at peer review.	Tom Zoeller
3. Nonparametric Reanalysis of thyroid histopathology in pups on PND5 from the Argus (1998a) neurodevelopmental study	Preliminary — Provided in response to request by Joe Haseman to correct some data entries and to extend analysis with more exact procedures	Joe Haseman Susan Porterfield Tom Zoeller
4. Hormone data for F0 and F1 generation in 2-generation reproductive study (Argus, 1998b).	Preliminary — These particular data are audited but the overall final report and data have not been audited nor released. Analysis represents alternative approach suggested by Joe Haseman.	Tom Zoeller Joe Haseman Susan Porterfield
5. Reproductive parameters (sperm morphology and estrous cyclicity) from F1 generation in 2-generation reproductive study (Argus, 1998b).	Preliminary — These particular data are audited but the overall final report and data have not been audited nor released.	Rochelle Tyl
6. Sheep red blood cell (SRBC) assays from 90-day experiments in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
7. Thyroid histopathology in mice from immunotoxicity studies	Preliminary — Data are audited but additional dose levels required for EPA to evaluate dose response	Tom Zoeller Susan Porterfield

Table 2. Data Analyses To Be Provided in February 8, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Occupational cross-sectional study of workers exposed via inhalation and an epidemiological study	Preliminary — Manuscripts submitted as accepted on 1/22/99. EPA analysis not complete.	Susan Porterfield Tom Zoeller Charles Emerson
2. Sheep red blood cell (SRBC) from 14-day experiment (repeat) in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
3. 14-day repeated dose pharmacokinetic study	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen
4. Correlations between percent of iodide uptake inhibition and hormone perturbations using single dose and repeated 14-day dose PK studies	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen Tom Zoeller

February 1, 1999 EPA Assessment Submission

Attachment #1
Final Genetox Review

A. Final NTP Salmonella battery
B. Repeat of Mouse Micronuclei assay by NTP
C. Review of A and B by EPA (Dellarco memo)
D. Repeat of Mouse Lymphoma by BioReliance
E. Review of D by EPA (Moore memo)
F. Revised section of document

ATTENTION PANEL MEMBER(S):

DAVID BRUSICK

January 28, 1999

NOTE TO: Annie Jarabek
FROM: Vicki Dellarco
RE: Review of the NTP Mutagenicity Studies on Ammonium Perchlorate

I have reviewed both the Ames assay and the mouse bone marrow micronucleus assay on ammonium perchlorate conducted under the auspices of the National Toxicology Program. Negative results were found in both assays. I find the protocols and the results from these tests to be acceptable. Furthermore, these recent studies confirm and reinforce the negative findings reported by another laboratory from these assays. I will revise the assessment document on perchlorate accordingly to reflect these new and important findings.

(NTP, 1999a)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: January 11, 1999

From: Errol Zeiger, Environmental Toxicology Program, NIEHS

Subject: Ammonium Perchlorate MN Summary Test Results

To: Annie Jarabek, National Center for Environmental Assessment, EPA

Male B6C3F1 mice were treated i.p with 125, 250, 500, 1000, 1500, and 2000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Five mice per group were injected daily for 3 consecutive days, and were sacrificed 24 hrs after the last injection. Their femoral bone marrow was removed and the polychromatic erythrocytes (PCE) scored for micronuclei (MN). All testing and scoring were done under code.

All animals in the 1500 and 2000 mg/kg groups died after the first i.p. injection, and 4/5 animals in the 1000 mg/kg group died after the second i.p. injection; the fifth animal was sacrificed and not scored for MN. All animals in the 125, 250, and 500 mg/kg groups survived the treatment; 2000 PCE's were scored per animal for MN.

The test data were analyzed statistically and have been entered into the NTP genetic toxicity database. No increases in MN-PCE were seen at any of the test doses, and the trend test was not positive. The positive control yielded a significant increase. No bone marrow toxicity was seen, as indicated by the percent PCE. The following table summarizes the results of the test.

mg/kg	mean MN cells/ 1000 PCE ± S.E.M.	pairwise p*	%PCE
0	3.00 ± 0.57		46.6
125	3.10 ± 0.40	0.4490	51.7
250	3.20 ± 0.56	0.3996	55.6
500	2.10 ± 0.29	0.8956	49.2
pos** 15	19.60 ± 2.03	0.0000	56.5

trend test p = 0.903

* p value for pairwise comparison against the solvent (0 dose) control
** positive control, cyclophosphamide

The results of this study are consistent with those reported in the Perchlorate Study Group report (Study No. 6100-001). In that study, which used gavage administration, the highest dose that could be scored was 1000 mg/kg.

(NTP, 1999b)

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Memorandum

Date: January 13, 1999
From: Errol Zeiger, Environmental Toxicology Program, NIEHS
Subject: Ammonium Perchlorate Salmonella Summary Test Results
To: Annie Jarabek, National Center for Environmental Assessment, EPA

The results of the NTP's Salmonella mutagenicity testing of Ammonium perchlorate are attached. The values presented are the means and standard errors of the mean, of triplicate plates.

The chemical was dissolved in water and tested using the preincubation procedure at doses from 100 to 10,000 µg/plate, without metabolic activation (NA), and using S-9 liver homogenates from Aroclor induced hamster (HLI) and rats (RLI). Two different concentrations of S-9 were used, 10% and 30%. The tests without metabolic activation (NA) were performed twice. Salmonella tester strains TA102, TA104, TA100, TA1535, TA97, and TA98 were used. "Pos" is the positive control.

Ammonium perchlorate was not toxic or mutagenic under the conditions of this test.

Although there were a number of differences between the NTP protocol and that used by the Perchlorate Study Group report (Study No. 6100-001), the conclusions of both tests are the same.

AMMONIUM PERCHLORATE

(LAB: SRI SOLVENT: H2O PROTOCOL: PREINC)

DOSE	TA102 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	163	12.1	205	13.7	312	14.8	269	10.5	274	29.3	281	8.4
100.000	182	8.2	207	25.0	319	17.1	270	21.1	302	9.5	275	11.0
333.000	174	4.5	220	14.2	316	10.5	257	16.5	306	14.7	262	5.8
1000.000	161	3.3	232	7.8	287	25.2	265	15.3	296	9.0	276	10.4
3333.000	182	6.0	216	9.5	291	10.0	240	18.7	271	7.3	265	23.7
10000.000	176	10.2	190	31.2	317	7.1	256	16.3	280	24.8	270	4.1
POS	751	27.7	739	17.1	1182	17.9	1049	44.5	1043	52.0	942	18.1

DOSE	TA104 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	247	12.7	317	20.7	436	12.4	334	15.7	422	23.6	334	17.3
100.000	280	9.7	341	17.6	439	18.7	344	21.5	404	16.7	310	11.3
333.000	254	26.3	318	18.8	374	60.4	373	8.7	426	8.2	344	17.6
1000.000	250	17.7	326	7.2	426	15.1	385	9.3	451	13.2	350	21.6
3333.000	272	15.1	338	19.3	424	12.7	351	13.7	413	18.6	344	27.6
10000.000	254	12.5	341	17.8	442	15.9	341	19.0	450	9.8	331	12.3
POS	847	25.7	843	28.0	1200	25.9	1260	12.5	962	18.6	1225	33.9

DOSE	TA100											
	NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	155	5.2	128	2.2	125	13.7	173	4.6	126	7.3	147	3.8
100.000	152	3.2	121	6.5	128	0.6	161	10.0	131	3.5	161	3.8
333.000	155	3.5	124	4.0	132	4.9	155	13.8	122	3.3	151	8.1
1000.000	163	4.7	128	13.8	133	4.5	164	3.0	133	6.2	148	6.0
3333.000	147	7.2	132	4.8	121	2.6	172	8.6	135	11.9	159	2.6
10000.000	157	14.1	126	6.1	119	4.9	170	5.5	126	2.9	146	4.9
POS	928	7.2	937	18.8	629	9.2	722	12.4	540	14.8	657	20.5

DOSE	TA1535											
	NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	12	2.2	9	1.8	16	3.2	9	1.8	15	1.5	11	1.5
100.000	13	0.6	9	1.8	13	3.5	10	0.7	16	0.9	15	2.7
333.000	10	1.3	14	1.9	11	0.9	14	3.2	9	2.1	12	0.3
1000.000	10	1.2	10	1.5	13	0.6	12	0.9	11	0.7	11	0.0
3333.000	13	3.3	12	1.2	10	1.5	12	0.9	12	1.7	13	1.9
10000.000	10	0.6	10	1.9	12	1.9	9	0.9	10	1.7	8	0.0
POS	835	18.2	856	11.6	152	8.7	131	8.4	137	8.7	110	6.7

DOSE	TA97 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	121	3.7	135	16.4	178	12.9	168	14.6	157	13.7	158	14.9
100.000	130	9.3	141	9.1	182	7.0	183	3.4	167	4.9	179	6.2
333.000	131	16.8	132	7.9	170	4.0	172	7.0	153	10.5	174	14.5
1000.000	140	7.4	161	3.7	162	6.4	191	3.1	149	8.1	168	5.8
3333.000	134	5.5	164	8.5	153	12.7	192	0.9	154	16.5	173	13.8
10000.000	124	4.0	122	6.3	177	12.4	131	9.9	143	11.3	167	5.2
POS	508	20.7	553	21.5	513	183.0	592	13.0	656	10.0	517	8.2

DOSE	TA98 NA (-)		NA (-)		10% HLI (-)		30% HLI (-)		10% RLI (-)		30% RLI (-)	
ug/PLATE	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0.000	22	4.1	24	3.0	25	3.8	17	1.8	27	3.8	24	1.3
100.000	17	1.7	29	5.9	35	3.3	22	2.1	32	3.2	23	2.4
333.000	17	0.9	21	3.8	26	2.8	20	2.5	29	0.9	24	3.0
1000.000	23	2.3	24	0.3	23	1.2	21	4.6	22	0.7	21	2.0
3333.000	18	3.5	21	1.5	30	2.0	17	3.8	35	3.8	20	3.1
10000.000	18	4.4	26	3.3	27	4.8	20	4.4	29	2.6	19	0.3
POS	355	17.6	362	7.7	543	12.9	545	16.9	466	18.2	536	45.1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
RESEARCH TRIANGLE PARK
NORTH CAROLINA 27711

MEMORANDUM

DATE: January 29, 1999

SUBJECT: Analysis of Perchlorate

FROM: Martha M. Moore, Chief (MD-68) Martha Moore
Genetic & Cellular Toxicology Branch

TO: Vicki Dellarco, (MD7509C)
Office of Pesticides Programs
Annie Jarabek, (MD-52)
Toxocologist

I have reviewed the mouse lymphoma data generated in the repeat analysis of perchlorate and based on this information, I am confident that the data are sufficient to determine the chemical to be nonmutagenic both with and without S9 activation. While I am a little concerned that the background mutant frequency is too low, particularly in the without S9 experiment, this data set looks overall to be very good. It is internally very consistent. The problems that were observed in the data generated by the first laboratory are not present in the data from this laboratory. The issue of low background mutant frequency relates to whether the laboratory is adequately quantitating all of the mutants. I think that the mutant colony sizing curves that are included in the data provides sufficient evidence that the laboratory is quantitating mutants properly.

BIORELIANCE
Formerly Microbiological Associates

BIORELIANCE CORPORATION
14920 BROSCHART ROAD
ROCKVILLE, MARYLAND 20850-3349 USA
PHONE: 301.738.1000 • FAX: 301.738.1036

January 27, 1999

Mr. Michael F. Girard
Perchlorate Study Group Representative
Highway 50 and Aerojet Road
Building 20019/Department 0330
Rancho Cordova, CA 95813-6000

Dear Mr. Girard:

Enclosed please find the original of the final report for the BioReliance study G98BA06.702, *In Vitro* Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay), which was performed using your test article: Ammonium perchlorate. Also enclosed is the Response to Audit Comments.

Should you require additional information or have questions, please call Dr. Richard San at (301) 738-1000, extension 2222.

Sincerely,

Diane Gray

Diane Gray
Secretary
Toxicology Testing Services

Enclosures

cc: Michael L. Dourson, Ph.D., DABT
Toxicology Excellence for Risk Assessment
4303 Hamilton Avenue
Cincinnati, OH 45223

Annie Jarabek (phone: 919-541-4847)
USEPA/NCEA
Progress Center
Catawba Building
3200 Highway 54
Research Triangle Park, NC 27709

R. San
P. Smith
Study file

Response to Audit Comments

Test Article ID: Ammonium perchlorate
MA Study No.: G98BA06.702
Report Type: Draft to Final

All changes requested by the Sponsor have been incorporated into the final report.

RS 1/27/99

FINAL REPORT

Study Title

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

Test Article

Ammonium perchlorate

Authors

Richard H. C. San, Ph.D.
Jane J. Clarke, B.A.

Study Completion Date

January 27, 1999

Performing Laboratory

BioReliance
9630 Medical Center Drive
Rockville, MD 20850

Laboratory Study Number

G98BA06.702

Sponsor

Perchlorate Study Group
Highway 50 and Aerojet Road
Building 20019/Department 0330
Rancho Cordova, CA 95813-6000

STATEMENT OF COMPLIANCE

Study G98BA06.702 was conducted in compliance with the US FDA Good Laboratory Practice Regulations as published in 21 CFR 58, the US EPA GLP Standards 40 CFR 160 and 40 CFR 792, the UK GLP Compliance Regulations, the Japanese GLP Regulations and the OECD Principles of Good Laboratory Practice in all material aspects with the following exceptions:

> The identity, strength, purity and composition or other characteristics to define the test or control article have not been determined by the testing facility.
>
> Analyses to determine the uniformity, concentration, or stability of the test or control mixtures were not performed by the testing facility.
>
> The stability of the test or control article under the test conditions has not been determined by the testing facility.

Richard H. C. San, Ph.D. — Study Director | Date: 1/27/99

QUALITY ASSURANCE STATEMENT

Study Title: *IN VITRO* MAMMALIAN CELL GENE MUTATION TEST

Study Number: G98BA06.702

Study Director: Richard H. C. San, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 04 DEC 98, TO STUDY DIR 04 DEC 98, TO MGMT 04 DEC 98
PHASE: Protocol Review

INSPECT ON 15 DEC 98, TO STUDY DIR 15 DEC 98, TO MGMT 17 DEC 98
PHASE: Dilution of test and/or control material

INSPECT ON 20 JAN 99-21 JAN 99, TO STUDY DIR 21 JAN 99, TO MGMT 22 JAN 99
PHASE: Draft Report

INSPECT ON 27 JAN 99, TO STUDY DIR 27 JAN 99, TO MGMT 27 JAN 99
PHASE: Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Diane B Madsen 1-27-99

Diane B. Madsen, B.S.
QUALITY ASSURANCE

DATE

In Vitro Mammalian Cell Gene Mutation Test
(L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

FINAL REPORT

Sponsor:	**Perchlorate Study Group** **Highway 50 and Aerojet Road** **Building 20019/Department 0330** **Rancho Cordova, CA 95813-6000**
Study Monitor:	**Michael F. Girard** **Perchlorate Study Group Representative**
Scientific Advisor:	**Michael L. Dourson, Ph.D., D.A.B.T.** **Toxicology Excellence for Risk Assessment**
Performing Laboratory:	**BioReliance** **9630 Medical Center Drive** **Rockville, MD 20850**
Test Article I.D.:	**ammonium perchlorate**
Test Article Lot No.:	**05006CQ**
Test Article Purity:	**99.999% (Provided by Sponsor)**
BioReliance Study No.:	**G98BA06.702**
Test Article Description:	**white, crystalline solid**
Storage Conditions:	**room temperature; protected from light and moisture**
Test Article Receipt:	**November 16, 1998**
Study Initiation:	**December 2, 1998**
Laboratory Manager:	**Jane J. Clarke, B.A.**

Study Director: Richard H. C. San, Ph.D. 1/27/99

Richard H. C. San, Ph.D. Date

TABLE OF CONTENTS

SUMMARY

The test article, ammonium perchlorate, was tested in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9. The preliminary toxicity assay was used to establish the dose range for the mutagenesis assay. The mutagenesis assay was used to evaluate the mutagenic potential of the test article.

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in DMSO at 500 mg/mL, the maximum concentration tested.

In the preliminary toxicity assay, the maximum concentration of ammonium perchlorate in treatment medium was 5000 µg/mL. No visible precipitate was present at any concentration in treatment medium. Selection of dose levels for the mutation assay was based on reduction of suspension growth relative to the solvent control. Substantial toxicity, i.e., suspension growth of ≤50% of the solvent control, was not observed at any concentration with or without S9 activation.

Based on the results of the preliminary toxicity assay, the doses chosen for the mutagenesis assay ranged from 50 to 5000 µg/mL for both the non-activated and S9-activated cultures. No visible precipitate was present at any concentration in treatment medium. No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10^6 clonable cells over that of the solvent control. There was not a dose-response trend. Toxicity in the cloned cultures, i.e., total growth of ≤50% of the solvent control, was not observed at any doses without activation but was observed with S9 activation at doses of 4000 and 5000 µg/mL.

The trifluorothymidine-resistant colonies for the positive and solvent control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

Under the conditions of this study, test article ammonium perchlorate was concluded to be negative in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells.

CHARACTERIZATION OF TEST AND CONTROL ARTICLES

The test article, ammonium perchlorate, was received by BioReliance on November 16, 1998 and was assigned the code number 98BA06. The test article was characterized by the manufacturer as a white powder, which should be stored in a cool dry place. Its purity was given as 99.999%. Upon receipt, the test article was described as a white, crystalline solid and was stored at room temperature, protected from light and moisture.

The vehicle (solvent) used to deliver ammonium perchlorate to the test system was DMSO (CAS 67-68-5) obtained from Fisher.

Methyl methanesulfonate (MMS), CAS 66-27-3, lot # 09419LR, expiration date 5/01, was supplied by Aldrich Chemical Company and was used as the positive control for the non-activated test system at stock concentrations of 1000 and 2000 µg/mL. 7,12-Dimethylbenz(a)anthracene (7,12-DMBA), CAS 57-97-6, lot # 85H0296, expiration date 1/99, was supplied by Sigma Chemical Company and was used at stock concentrations of 250 and 400 µg/mL as the positive control for the S9-activated test system.

MATERIALS AND METHODS

Test System

L5178Y cells, clone 3.7.2C, were obtained from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, NC. Each lot of cryopreserved cells was tested using the agar culture and Hoechst staining procedures and found to be free of mycoplasma contamination. Prior to use in the assay, L5178Y cells were cleansed of spontaneous $TK^{-/-}$ cells by culturing in a restrictive medium (Clive and Spector, 1975).

Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor-1254, 500 mg/kg, five days prior to sacrifice. The S9 was batch prepared and stored at ≤-70°C until used. Each bulk preparation of S9 was assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(a)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 was mixed with the cofactors and Fischer's Medium for Leukemic Cells of Mice with 0.1% Pluronics (F_0P) to contain 250 µL S9, 6.0 mg nicotinamide adenine dinucleotide phosphate (NADP), 11.25 mg DL-isocitric acid and 750 µL F_0P per mL of S9-activation mixture and kept on ice until used. The cofactor/F_0P mixture was filter sterilized and adjusted to pH 7.0 prior to the addition of S9. The formulation of the activation mixture is based on information from Turner *et al.* (1984). The final concentration of S9 in the treatment medium was 10%.

Solubility Test

A solubility test was conducted to select the solvent. The test was conducted using one or more of the following solvents in the order of preference as listed: distilled water, dimethyl sulfoxide, ethanol and acetone. The test article was tested to determine the solvent, selected in order of preference, that permitted preparation of the highest soluble or workable concentration, up to 500 mg/mL (the highest concentration tested).

Preliminary Toxicity Assay

The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.5 to 5000 µg/mL in both the absence and presence of S9-activation.

Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to $3x10^5$ cells/mL after 24 hours only. Cultures with less than $3x10^5$ cells/mL were not adjusted. Toxicity was measured as suspension growth relative to the growth of the solvent controls.

Mutagenesis Assay

The mutagenesis assay was used to evaluate the mutagenic potential of the test article. L5178Y mouse lymphoma cells were exposed to the solvent alone and at least eight concentrations of test article in duplicate in both the absence and presence of S9. Positive controls, with and without S9-activation, were tested concurrently.

Treatment of the Target Cells

The mutagenesis assay was performed according to a protocol described by Clive and Spector (1975). Treatment was carried out in conical tubes by combining 6 x 106 L5178Y/TK+/- cells, 4 mL F0P medium or S9 activation mixture and 100 µL dosing solution of test or control article in solvent or solvent alone in a total volume of 10 mL. A total of at least eight concentrations of test article were tested in duplicate. The positive controls were treated with MMS (at final concentrations in treatment medium of 10 and 20 µg/mL) and 7,12-DMBA (at final concentrations in treatment medium of 2.5 and 4.0 µg/mL). Treatment tubes were gassed with 5±1% CO2 in air, capped tightly, and incubated with mechanical mixing for 4 hours at 37±1°C.

The preparation and addition of the test article dosing solutions were carried out under amber lighting and the cells were incubated in the dark during the exposure period. After the treatment period, the cells were washed twice with F0P or F0P supplemented with 10% horse serum and 2 mM L-glutamine (F10P). After the second wash, the cells were resuspended in F10P, gassed with 5±1% CO2 in air and placed on the roller drum apparatus at 37±1°C.

Expression of the Mutant Phenotype

For expression of the mutant phenotype, the cultures were counted using an electronic cell counter and adjusted to $3x10^5$ cells/mL at approximately 24 and 48 hours after treatment in 20 and 10 mL total volume, respectively. Cultures with less than $3x10^5$ cells/mL were not adjusted.

For expression of the $TK^{-/-}$ cells, cells were placed in cloning medium (C.M.) containing 0.23% granulated agar. Two flasks per culture to be cloned were labeled with the test article concentration, activation condition, and either TFT (trifluorothymidine, the selective agent) or V.C. (viable count). Each flask was prewarmed to 37±1°C, filled with 100 mL C.M., and placed in an incubator shaker at 37±1°C until used. The cells were centrifuged at 1000 rpm for 10 minutes and the supernatant was decanted. The cells were then diluted in C.M. to concentrations of $3x10^6$ cells/100 mL C.M. for the TFT flask and 600 cells/100 mL C.M. for the V.C. flask. After the dilution, 1.0 mL of stock solution of TFT was added to the TFT flask (final concentration of 3 μg/mL) and both this flask and the V.C. flask were placed on the shaker at 125 rpm and 37±1°C. After 15 minutes, the flasks were removed and 33 mL of the cell suspension was pipetted into each of three appropriately labeled petri dishes. To accelerate the gelling process, the plates were placed in cold storage (approximately 4°C) for approximately 30 minutes. The plates were then incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for 10-14 days.

Scoring Procedures

After the incubation period, the V.C. plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were then counted for each culture with ≥10% total relative growth. The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm. The rationale for this procedure is as follows: Mutant L5178Y $TK^{-/-}$ colonies exhibit a characteristic frequency distribution of colony sizes. The precise distribution of large and small TFT-resistant mutant colonies appears to be the characteristic mutagenic "finger-print" of carcinogens in the L5178Y $TK^{+/-}$ system (Clive *et al.*, 1979; DeMarini *et al.*, 1989). Clive *et al.* (1979) and Hozier *et al.* (1981) have presented evidence to substantiate the hypothesis that the small colony variants carry chromosome aberrations associated with chromosome 11, the chromosome on which the TK locus is located in the mouse (Kozak and Ruddle, 1977). They suggested that large colony mutants received very localized damage, possibly in the form of a point mutation or small deletion within the TK locus, while small colony mutants received damage to collateral loci concordant with the loss of TK activity.

Evaluation of Results

The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 10^6 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding V.C. plates and multiplying by the dilution factor (2×10^{-4}) then multiplying by 10^6. For simplicity, this is described as: (Average # TFT colonies / average # VC colonies) x 200 in the tables.

In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. All conclusions were based on sound scientific judgement; however, the following criteria are presented as a guide to interpretation of the data (Clive *et al.*, 1995):

- The result was considered to induce a positive response if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level.

- A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level.

- Test articles producing fewer than 55 mutants per 10^6 clonable cells over the background level were concluded to be negative.

Criteria for a Valid Test

The following criteria must be met for the mutagenesis assay to be considered valid:

Negative Controls:

The spontaneous mutant frequency of the solvent control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%.

Positive Controls:

At least one concentration of each positive control must exhibit mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).

Test Article-Treated Cultures:

A minimum of four analyzable concentrations with mutant frequency data will be required.

Archives

All raw data, protocol, and a copy of all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at:

BioReliance
14920 Broschart Rd.
Rockville, MD 20850

RESULTS AND DISCUSSION

Solubility Test

Dimethyl sulfoxide (DMSO) was selected as the solvent of choice based on solubility of the test article and compatibility with the target cells. The test article was soluble in DMSO at 500 mg/mL, the maximum concentration tested.

Preliminary Toxicity Assay

The results of the preliminary toxicity assay are presented in Table 1. The maximum dose tested in the preliminary toxicity assay was 5000 µg/mL. No visible precipitate was present at any dose level in treatment medium. The osmolality of the solvent control was 447 mmol/kg and the osmolality of the highest soluble dose, 5000 µg/mL, was 462 mmol/kg. Suspension growth relative to the solvent controls was 89% at 5000 µg/mL without activation and 72% at 5000 µg/mL with S9 activation. Based on the results of the toxicity test, the doses chosen for the mutagenesis assay ranged from 50 to 5000 µg/mL for both the non-activated and S9-activated cultures.

Mutagenesis Assay

The results of the mutagenesis assay are presented in Tables 2 through 5. Colony size distributions are presented in Figures 1 and 2. No visible precipitate was present at any dose level in treatment medium. In the non-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL were cloned and produced a range in suspension growth of 61% to 98%. In the S9-activated system, cultures treated with concentrations of 1000, 2000, 3000, 4000 and 5000 µg/mL were cloned and produced a range in suspension growth of 14% to 80%.

No cloned cultures exhibited mutant frequencies that were at least 55 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed in the non-activated or S9-activated systems. The total growths ranged from 69% to 92% for the non-activated cultures at concentrations of 1000 to 5000 μg/mL and 13% to 85% for the S9-activated cultures at concentrations of 1000 to 5000 μg/mL.

The TFT-resistant colonies for the positive and solvent control cultures were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, ammonium perchlorate was concluded to be negative.

REFERENCES

Aaron, C.S., Bolcsfoldi, G., Glatt, H.-R., Moore, M., Nishi, Y., Stankowski, L., Theiss, J. and Thompson, E. (1994) Mammalian cell gene mutation assays working group report. Mutation Res. 312:235-239.

Clive, D., Bolcsfoldi, G., Clements, J., Cole, J., Homna, M., Majeska, J., Moore, M., Muller, L., Myhr, B., Oberly, T., Oudelhkim, M., Rudd, C., Shimada, H., Sofuni, T., Thybaud, V. and Wilcox, P. (1995) Consensus agreement regarding protocol issues discussed during the mouse lymphoma workshop: Portland, Oregon, May 7, 1994. Environ. Molec. Mutagen. 25:165-168.

Clive D., Johnson K.O., Spector J.F.S., Batson A.G. and Brown M.M.M. (1979) Validation and characterization of the L5178Y $TK^{+/-}$ mouse lymphoma mutagen assay system. Mutation Res. 59:61-108.

Clive, D. and Spector, J.F.S. (1975) Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178 $TK^{+/-}$ mouse lymphoma cells. Mutation Res. 31:17-29.

DeMarini, D.M., Brockman, H.E., de Serres, F.J., Evans, H.H., Stankowski Jr, L.F. and Hsie, A.W. (1989) Specific-locus mutations induced in eukaryotes (especially mammalian cells) by radiation and chemicals: a perspective. Mutation Res. 220:11-29.

Hozier, J., Sawyer, J., Moore, M., Howard, B. and Clive, D. (1981) Cytogenetic analysis of the L5178Y/$TK^{+/-}$ $\rightarrow$ $TK^{-/-}$ mouse lymphoma mutagenesis assay system. Mutation Res. 84:169-181.

Kozak, C.A. and Ruddle, F.H. (1977) Assignment of the genes for thymidine kinase and galactokinase to *Mus musculus* chromosome 11 and the preferential segregation of this chromosome in Chinese hamster/mouse somatic cell hybrids. Somatic Cell Genet. 3:121-133.

Moore, M.M., Clive, D., Howard, B.E., Batson, A.G. and Turner, N.T. (1985) In situ analysis of trifluorothymidine-resistant (TFT') mutants of L5178Y/$TK^{+/-}$ mouse lymphoma cells. Mutation Res. 151:147-159.

Turner, N.T., Batson, A.G. and Clive, D. (1984) Procedures for the L5178Y/$TK^{+/-}$ $\rightarrow$ $TK^{-/-}$ Mouse Lymphoma Cell Mutagenicity Assay. In: B.J. Kilbey, M. Legator, W. Nichols and C. Ramel (Eds.), Handbook of Mutagenicity Test Procedures, Second Edition, Elsevier, Amsterdam, pp. 239-268.

TABLE 1

PRELIMINARY TOXICITY ASSAY USING ammonium perchlorate

Test Article Concentration (µg/mL)	Cell Concentration (X10^6)[a] Day 1	Day 2	Suspension Growth Total[b]	% of Control[c]
WITHOUT ACTIVATION				
Solvent 1	0.921	1.343	13.7	
Solvent 2	0.915	1.297	13.2	
.5	0.901	1.362	13.6	101
1.5	0.923	1.357	13.9	103
5	0.863	1.373	13.2	98
15	0.827	1.392	12.8	95
50	0.872	1.323	12.8	95
150	0.926	1.282	13.2	98
500	0.862	1.359	13.0	97
1500	0.895	1.281	12.7	95
5000	0.732	1.469	11.9	89
WITH S-9 ACTIVATION				
Solvent 1	0.663	1.292	9.5	
Solvent 2	0.650	1.333	9.6	
.5	0.686	1.368	10.4	109
1.5	0.693	1.379	10.6	111
5	0.700	1.349	10.5	110
15	0.652	1.307	9.5	99
50	0.663	1.341	9.9	103
150	0.647	1.316	9.5	99
500	0.661	1.333	9.8	102
1500	0.606	1.413	9.5	99
5000	0.507	1.224	6.9	72

Solvent = DMSO

1 and 2 are duplicate cultures

[a] - Cultures containing <0.3×10^6 cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3×10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts			TFT Mean	VC Colonies Counts			VC Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	22	21	24	22 ±1	191	178	170	180 ±9	25		
Solvent	2	20	17	25	21 ±3	179	140	172	164 ±17	25		
Mean Solvent Mutant Frequency= 25												
1000	A	27	17	15	20 ±5	149	176	148	158 ±13	25	0	83
1000	B	13	21	24	19 ±5	157	156	131	148 ±12	26	1	84
2000	A	28	26	14	23 ±6	168	155	159	161 ±5	28	3	81
2000	B	18	22	19	20 ±2	172	181	169	174 ±5	23	-2	92
3000	A	25	25	25	25 ±0	172	158	163	164 ±6	30	5	90
3000	B	24	24	26	25 ±1	153	177	181	170 ±12	29	4	85
4000	A	19	14	14	16 ±2	190	179	197	189 ±7	17	-8	86
4000	B	31	20	24	25 ±5	205	184	207	199 ±10	25	0	92
5000	A	17	24	25	22 ±4	195	183	157	178 ±16	25	0	69
5000	B	24	34	32	30 ±4	203	188	189	193 ±7	31	6	69
Positive Control - Methyl Methanesulfonate (µg/mL)												
10		106	139	159	135 ±22	114	110	114	113 ±2	239	214	48
20		106	127	119	117 ±9	35	40	46	40 ±4	582	557	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

TABLE 3

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK+/- MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.438	1.362	21.8		180		
Solvent	2	1.446	1.270	20.4		164		
1000	A	1.229	1.397	19.1	90	158	92	83
1000	B	1.301	1.425	20.6	98	148	86	84
2000	A	1.236	1.333	18.3	87	161	94	81
2000	B	1.311	1.306	19.0	90	174	101	92
3000	A	1.200	1.492	19.9	94	164	96	90
3000	B	1.187	1.367	18.0	85	170	99	85
4000	A	1.131	1.318	16.6	79	189	110	86
4000	B	1.135	1.323	16.7	79	199	116	92
5000	A	1.062	1.193	14.1	67	178	104	69
5000	B	1.068	1.085	12.9	61	193	113	69
Positive Control - Methyl Methanesulfonate (µg/mL)								
10		1.232	1.134	15.5	74	113	66	48
20		1.081	0.884	10.6	50	40	23	12

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Cultures containing $<0.3\times10^6$ cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / 0.3×10^6 cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

TABLE 4

CLONING DATA FOR L5178Y/TK+/- MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts			TFT Mean	VC Colonies Counts			VC Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	21	28	30	26 ±4	150	130	157	146 ±11	36		
Solvent	2	24	34	50	36 ±11	163	178	163	168 ±7	43		
Mean Solvent Mutant Frequency= 40												
1000	A	24	30	18	24 ±5	186	208	166	187 ±17	26	-14	80
1000	B	15	19	28	21 ±5	158	157	181	165 ±11	25	-15	85
2000	A	35	35	25	32 ±5	190	173	153	172 ±15	37	-3	65
2000	B	21	24	26	24 ±2	199	170	207	192 ±16	25	-15	82
3000	A	35	38	33	35 ±2	192	165	169	175 ±12	40	1	58
3000	B	28	22	34	28 ±5	186	176	186	183 ±5	31	-9	64
4000	A	38	32	33	34 ±3	169	172	150	164 ±10	42	2	41
4000	B	33	28	34	32 ±3	183	185	175	181 ±4	35	-5	42
5000	A	38	34	47	40 ±5	135	137	142	138 ±3	57	18	13
5000	B	+	40	50	45 ±4	191	162	173	175 ±12	51	12	21
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)												
2.5		135	129	136	133 ±3	120	129	137	129 ±7	207	168	65
4		171	166	189	175 ±10	130	111	99	113 ±13	309	270	42

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

+ - Culture lost to contamination

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

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TABLE 5

TOTAL COMPOUND TOXICITY DATA FOR L5178Y/TK+/- MOUSE LYMPHOMA CELLS TREATED WITH ammonium perchlorate IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		Cell Concentration (X 10^6)[a] Day 1	Day 2	Susp Growth Total[b]	%Cntl[c]	Cloning Growth Avg VC	%Cntl[d]	% Total Growth[e]
Solvent	1	1.291	1.443	20.7		146		
Solvent	2	1.315	1.503	22.0		168		
1000	A	1.150	1.129	14.4	68	187	119	80
1000	B	1.225	1.256	17.1	80	165	105	85
2000	A	1.014	1.114	12.5	59	172	110	65
2000	B	1.060	1.218	14.3	67	192	122	82
3000	A	0.919	1.088	11.1	52	175	112	58
3000	B	0.845	1.248	11.7	55	183	116	64
4000	A	0.685	1.108	8.4	40	164	104	41
4000	B	0.642	1.083	7.7	36	181	115	42
5000	A	0.293	0.919	3.1	14	138	88	13
5000	B	0.412	0.866	4.0	19	175	112	21
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)								
2.5		1.087	1.397	16.9	79	129	82	65
4		0.949	1.188	12.5	59	113	72	42

Solvent = DMSO

A and B or 1 and 2 are duplicate cultures

[a] - Cultures containing $<0.3x10^6$ cells/mL on day 1 and 2 are considered as having 0% total suspension growth.

[b] - Total suspension growth = (Day 1 cell conc. / $0.3x10^6$ cells/mL) x (Day 2 cell conc. / Day 1 adjusted cell conc.)

[c] - % of control suspension growth = (total treatment suspension growth / average solvent control total suspension growth) x 100

[d] - % control cloning growth = (average V.C. of treated culture / average V.C. of solvent control) x 100

[e] - % total growth = (% suspension growth x % cloning growth) / 100

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Figure 1

Colony Size Distribution in the Absence of Metabolic Activation

(Positive Control Compared with Solvent Control)

G98BA06.702 B1 MMS

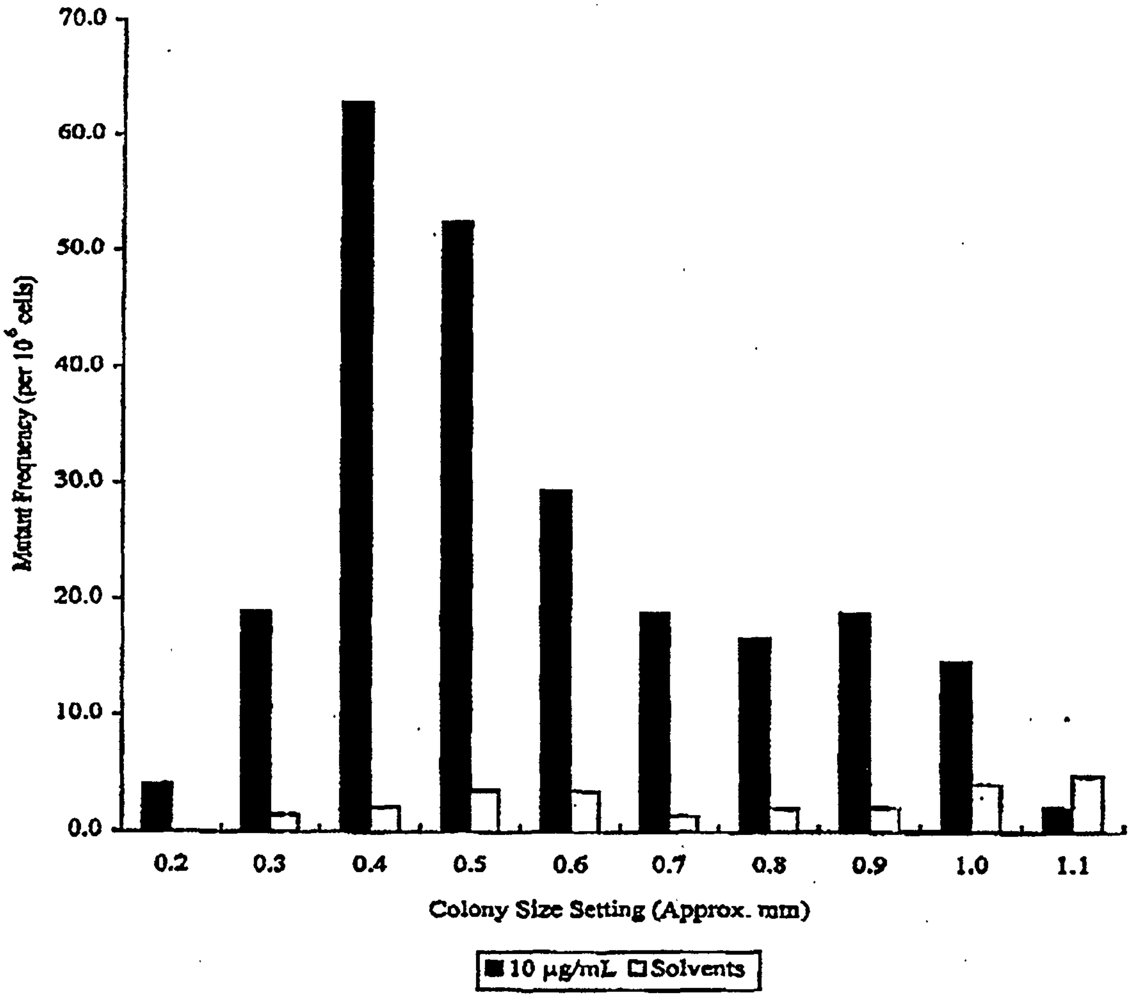

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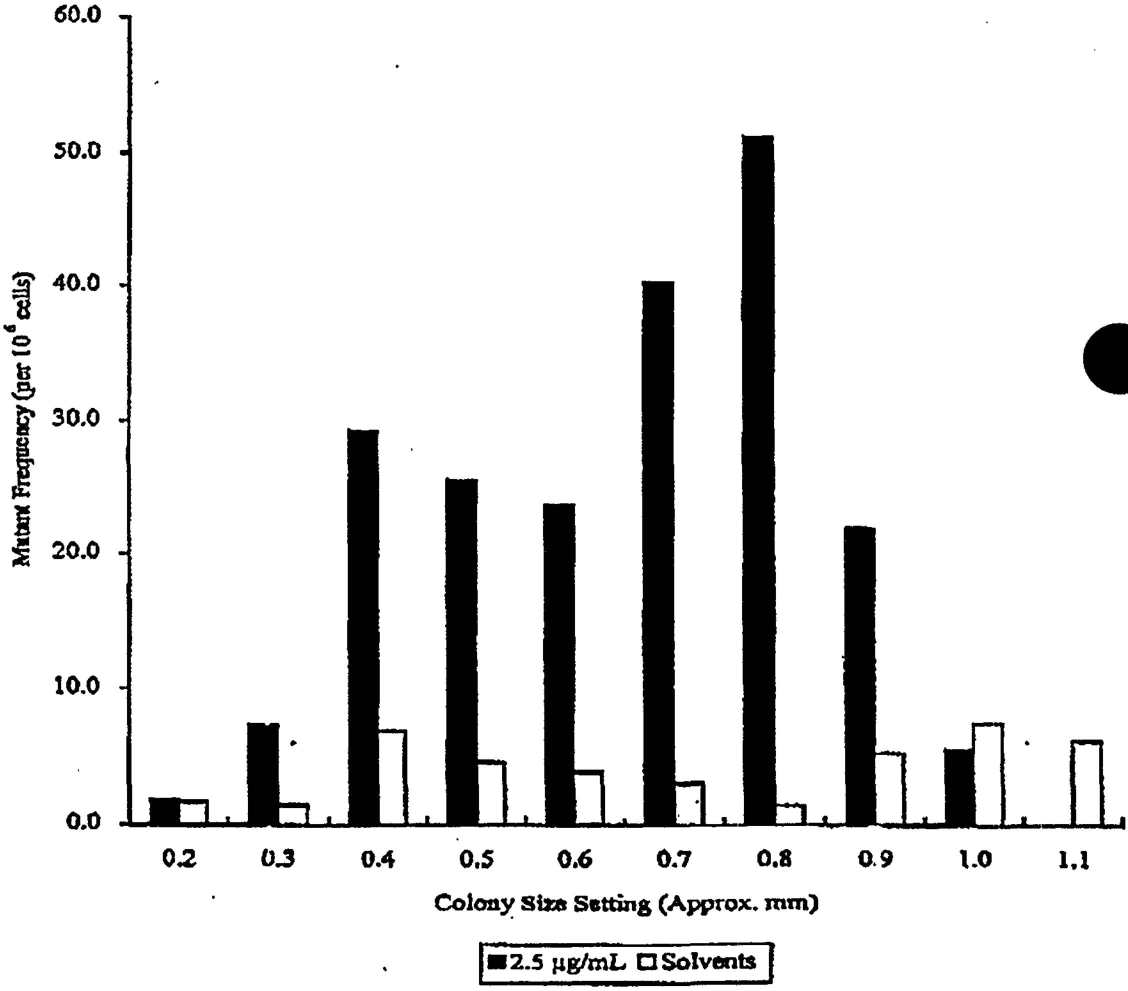
Figure 2

Colony Size Distribution in the Presence of Metabolic Activation

(Positive Control Compared with Solvent Control)

G98BA06.702 B1 DMBA



APPENDIX I

Historical Control Data

Mouse Lymphoma Historical Control Data

1995-1997

	Non-activated			S9-Activated		
	Solvent Control	20 µg/mL MMS	10 µg/mL MMS	Solvent Control	4.0µg/mL DMBA	2.5µg/mL DMBA
Mean MF	35.7	655.3	336.0	58.0	453.2	269.8
SD	10.3	293.3	128.5	18.6	158.5	95.1
Maximum	68.0	2400.0	729.0	100.0	1029.0	1048.0
Minimum	20.0	198.0	128.0	28.0	222.0	141.0

Solvent control (Fischer's medium, distilled water, saline, DMSO, ethanol, acetone or vehicle supplied by Sponsor)

MMS Methyl methanesulfonate
DMBA Dimethylbenz(a)anthracene
MF Mutant frequency per 10^6 clonable cells
SD Standard deviation

APPENDIX II

Study Protocol

QA APPROVED B6M 12-4-98

Received by RA/QA 12/02/98

BioReliance Study Number: G98BA06.702

In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay)

1.0 PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test article based on quantitation of forward mutations at the thymidine kinase locus of L5178Y mouse lymphoma cells.

2.0 SPONSOR

2.1	Name:	**Perchlorate Study Group**
2.2	Address:	Highway 50 and Aerojet Road Building 20019/Department 0330 Rancho Cordova, CA 95813-6000
2.3	Study Monitor:	Michael F. Girard Perchlorate Study Group Representative Telephone: (916) 355-2945 Telefax: (916) 355-6145
2.4	Scientific Advisor:	Michael L. Dourson, Ph.D., DABT Toxicology Excellence for Risk Assessment 4303 Hamilton Ave. Cincinnati, OH 45223 Telephone: (513) 542-7475 Telefax: (513) 542-7487
2.5	Sponsor Project #:	NP

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1	Test Article:		**Ammonium perchlorate**
3.2	Controls:	Negative:	Test article solvent (or vehicle)
		Positive:	Methyl methanesulfonate (MMS) 7,12-dimethylbenz(a)anthracene (DMBA)

3.3 Determination of Strength, Purity, etc.

Unless alternate arrangements are made, the testing facility at BioReliance will not perform analysis of the dosing solutions. The Sponsor will be directly responsible for determination and documentation of the analytical purity and composition of the test article, and the stability and strength of the test article in the solvent (or vehicle).

3.4 Test Article Retention Sample

The retention of a reserve sample of the test article will be the responsibility of the Sponsor.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1 Name: Toxicology Testing Facility
BioReliance

4.2 Address: 9630 Medical Center Drive
Rockville, MD 20850

4.3 Study Director: Richard H. C. San, Ph.D.

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 12/7/98

5.2 Proposed Experimental Completion Date: 1/19/99

5.3 Proposed Report Date: 1/28/99

6.0 TEST SYSTEM

L5178Y/TK$^{+/-}$ mouse lymphoma cells are heterozygous at the normally diploid thymidine kinase (TK) locus. L5178Y/TK$^{+/-}$, clone 3.7.2C, were received from Patricia Poorman-Allen, Glaxo Wellcome Inc., Research Triangle Park, North Carolina. Each freeze lot of cells has been tested and found to be free of mycoplasma contamination. This system has been demonstrated to be sensitive to the mutagenic activity of a variety of chemicals.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The mammalian mutation assay will be performed by exposing duplicate cultures of L5178Y/TK$^{+/-}$ cells to a minimum of eight concentrations of test article as well as positive and negative (solvent) controls. Exposures will be for 4 hours in the presence and absence of an S9 activation system. Following a two day expression period, with daily cell population adjustments, cultures demonstrating 0% to 90% growth inhibition will be cloned, in triplicate, in restrictive medium containing soft agar to select for the mutant phenotype. After a 10 to 14 day selection period, mutant colonies will be enumerated. The mutagenic potential of the test article will be measured by its ability to induce TK$^{+/-}$ $\rightarrow$ TK$^{-/-}$ mutations. For those test articles demonstrating a positive response, mutant colonies will be sized as an indication of mechanism of action.

7.1 Selection of Solvent

Unless the Sponsor has indicated the test article solvent, a solubility determination will be conducted to measure the maximum soluble concentration in a variety of solvents. Solvents compatible with this test system, in order of preference, include, but are not limited to, culture medium or distilled water (CAS 7732-18-5), dimethyl sulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5) and acetone CAS 67-64-1). The solvent of choice will be that solvent, selected in order of preference, that permits preparation of the highest soluble stock concentration, up to a maximum of 500 mg/ml.

7.2 Dose Selection

In the preliminary toxicity test, L5178Y/TK$^{+/-}$ cells will be exposed to solvent alone and to at least nine concentrations of test article, the highest concentration being the lowest insoluble dose in treatment medium but not to exceed 5000 µg/ml. The pH of the treatment medium will be adjusted, if necessary, to maintain a neutral pH in the treatment medium. The osmolality of the highest soluble treatment condition will also be measured. After a 4-hour treatment in the presence and absence of S9 activation, cells will be washed twice with F_0P (Fischer's Media for Leukemic Cells of Mice with 0.1% Pluronics) or $F_{10}P$ (F_0P supplemented with 10% horse serum and 2mM L-glutamine) and cultured in suspension for two days post-treatment, with cell concentration adjustment on the first day.

Selection of dose levels for the mutation assay will be based on reduction of suspension growth after treatment in the preliminary toxicity test. Unless specified otherwise by the Sponsor, the high dose for the mutation assay will be that concentration exhibiting approximately 100% growth inhibition. The low dose will be selected to exhibit 0% growth inhibition. For freely soluble, non-toxic test articles, the highest concentration will be 5000 µg/ml. For relatively insoluble, non-toxic test articles, the highest concentration will be the lowest insoluble dose in treatment medium but not to exceed 5000 µg/ml. In all cases, precipitation will be evaluated at the beginning and at the end of the treatment period using the naked eye (ICH, 1996).

7.3 Route and Frequency of Administration

Cell cultures will be treated for 4 hours by way of a vehicle compatible with the system, both in the presence and absence of metabolic activation. This technique of administration has been demonstrated to be effective in the detection of chemical mutagens in this system.

7.4 Exogenous Metabolic Activation

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The source of S9 will be adult male Sprague-Dawley rats induced by a single injection of Aroclor 1254 at a dose level of 500 mg/kg body weight five days prior to sacrifice. The S9 will be batch prepared and stored frozen at approximately -70°C until used.

Immediately prior to use. the S9 will be thawed and mixed with a cofactor pool to contain 11.25 mg DL-isocitric acid, 6 mg NADP, and 0.25 ml S9 homogenate per ml in F_0P. The S9 mix will be adjusted to pH 7.

7.5 Controls

7.5.1 Negative Control

The solvent (or vehicle) for the test article will be used as the negative control.

7.5.2 Positive Controls

Methyl methanesulfonate (MMS) will be used at two concentrations of 10 and 20 μg/ml as the positive control for the non-activated test system. For the S9-activated system, 7,12-dimethylbenz(a)anthracene (DMBA) will be used at two concentrations of 2.5 and 4.0 μg/ml.

7.6 Preparation of Target Cells

Prior to use in the assay, L5178Y/$TK^{+/-}$ cells will be cleansed to reduce the frequency of spontaneously occurring $TK^{-/-}$ cells. Using the procedure described by Clive and Spector (1975), L5178Y cells will be cultured for 24 hours in the presence of thymidine, hypoxanthine, methotrexate and glycine to poison the $TK^{-/-}$ cells.

L5178Y/$TK^{+/-}$ cells will be prepared at 1 x 10^6 cells/ml in 50% conditioned $F_{10}P$ and 50% F_0P. If cultures are to be treated with more than 100 μl of test article dosing solution, the cell concentration may be adjusted.

7.7 Identification of the Test System

Using a permanent marking pen, the treatment tubes will be identified by the study number and a code system to designate the treatment condition and test phase.

7.8 Treatment of Target Cells

Treatment will be carried out in conical tubes by combining 100 μl dosing solution of test or control article in solvent or solvent alone, 4 ml F_0P medium or S9 activation mixture with 6 x 10^6 L5178Y/$TK^{+/-}$ cells in a total volume of 10 ml. A minimum of eight concentrations of test article will be tested in duplicate. All pH adjustments will be performed prior to adding S9 or target cells to the treatment medium. Volumes of test article dosing solution in excess of 100 μl may be used if required to achieve the target final concentration in treatment medium. Treatment tubes will be gassed with 5±1% CO_2 in air. capped tightly. and incubated with mechanical mixing for 4 hours at 37±1°C. The preparation and addition of the test article dosing solutions will be carried out under amber lighting and the cells will be incubated in the dark during the 4-hour exposure period.

7.9 Expression of the Mutant Phenotype

At the end of the exposure period, the cells will be washed twice with F_0P or $F_{10}P$ and collected by centrifugation. The cells will be resuspended in 20 ml $F_{10}P$, gassed with 5±1% CO_2 in air and cultured in suspension at 37±1°C for two days following treatment. Cell population adjustments to 0.3 x 10^6 cells/ml will be made at 24 and 48 hours.

7.10 Selection of the Mutant Phenotype

For selection of the trifluorothymidine (TFT)-resistant phenotype, cells from a minimum of five non-activated and five S9-activated test article concentrations demonstrating from 0% to 90% suspension growth inhibition will be plated into three replicate dishes at a density of 1 x 10^6 cells/100mm plate in cloning medium containing 0.23% agar and 2-4 µg TFT/ml. For estimation of cloning efficiency at the time of selection, 200 cells/100mm plate will be plated in triplicate in cloning medium free of TFT (viable cell (VC) plate). Plates will be incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 for 10-14 days.

The total number of colonies per plate will be determined for the VC plates and the total relative growth calculated. The total number of colonies per TFT plate will then be determined for those cultures with ≥10% total growth. Colonies are enumerated using an automatic counter; if the automatic counter cannot be used, the colonies will be counted manually. The diameters of the TFT colonies from the positive control and solvent control cultures will be determined over a range of approximately 0.2 to 1.1 mm. In the event the test article demonstrates a positive response, the diameters of the TFT colonies for at least one dose level of the test article (the highest positive concentration) will be determined over a range of approximately 0.2 to 1.1 mm.

7.11 Independent Repeat Assay

Verification of a clear positive response will not be required (OECD Guideline 476, ICH 1997). For equivocal and negative results, the Sponsor will be consulted regarding the need for an independent repeat assay.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

8.1 Negative Controls

The spontaneous mutant frequency of the solvent (or vehicle) control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent (or vehicle) control group must be greater than 50%.

8.2 Positive Controls

At least one concentration of each positive control must exhibit mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).

8.3 Test Article-Treated Cultures

A minimum of four analyzable concentrations with mutant frequency data will be required.

9.0 EVALUATION OF TEST RESULTS

The cytotoxic effects of each treatment condition are expressed relative to the solvent-treated control for suspension growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency for each treatment condition is calculated by dividing the mean number of colonies on the TFT-plates by the mean number of colonies on the VC-plates and multiplying by the dilution factor (2×10^{-4}), and is expressed as TFT-resistant mutants per 10^6 surviving cells.

In evaluation of the data, increases in mutant frequencies which occur only at highly toxic concentrations (i.e., less than 10% total growth) are not considered biologically relevant. All conclusions will be based on sound scientific judgement; however, the following criteria are presented as a guide to interpretation of the data (Clive *et al.*, 1995):

- The result will be considered to induce a positive response if a concentration-related increase in mutant frequency is observed and one or more dose levels with 10% or greater total growth exhibit mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level.

- A result will be considered equivocal if the mutant frequency in treated cultures is between 55 and 99 mutants per 10^6 clonable cells over the background level.

- Test articles producing fewer than 55 mutants per 10^6 clonable cells over the background level will be concluded to be negative.

10.0 REPORT

A report of the results of this study will be prepared by the Testing Laboratory and will accurately describe all methods used in the generation and analysis of data.

Results presented will include, but not be limited to:

- test substance: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test article, if known.
- solvent/vehicle: justification for choice of vehicle; solubility and stability of test article in solvent/vehicle, if known.
- cell type used, number of cultures, methods for maintenance of cell cultures
- rationale for selection of concentrations and number of cultures
- test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period, selective agent
- method used to enumerate numbers of viable and mutant colonies and the number of colonies in each plate
- dose-response relationship, if applicable
- distribution of the mutant colony diameter for the solvent and positive controls and, when the test article induces a positive response, for at least one dose level of the test article (the highest positive concentration)
- positive and solvent control historical data

11.0 RECORDS AND ARCHIVES

Upon completion of the final report, all raw data and reports will be maintained in the archives of BioReliance, Rockville, MD in accordance with the relevant Good Laboratory Practice Regulations.

12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), July 1997, and with the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202. April 24, 1996.

This study will be performed in compliance with the provisions of the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.

Will this study be submitted to a regulatory agency? yes

If so, to which agency or agencies? U.S. EPA, U.S DOD

Unless arrangements are made to the contrary, unused dosing solutions will be disposed of following administration to the test system and all residual test article will be disposed of following finalization of the report.

13.0 REFERENCES

Aaron, C.S., Bolcsfoldi, G., Glatt, H.-R., Moore, M., Nishi, Y., Stankowski, L., Theiss. J. and Thompson, E. (1994) Mammalian cell gene mutation assays working group report. Mutation Research 312:235-239.

Clive, D., Bolcsfoldi, G., Clements, J., Cole, J., Homna, M., Majeska, J., Moore, M., Muller, L., Myhr, B., Oberly, T., Oudelhkim, M., Rudd, C., Shimada, H., Sofuni, T., Thybaud, V. and Wilcox, P. (1995) Consensus agreement regarding protocol issues discussed during the mouse lymphoma workshop: Portland, Oregon, May 7, 1994. Environ. Molec. Mutagen. 25:165-168.

Clive, D. and Spector, J.F.S. (1975) Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Research 31:17-29.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals. S2A document recommended for adoption at step 4 of the ICH process on July 19, 1995. Federal Register 61:18198-18202. April 24, 1996.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals. S2B document recommended for adoption at step 4 of the ICH process on July 16, 1997. Federal Register 62:16026-16030, November 21, 1997.

Moore, M.M., Clive, D., Howard, B.E., Batson, A.G. and Turner, N.T. In situ analysis of trifluorothymidine-resistant (TFT) mutants of L5178Y/TK$^{+/-}$ mouse lymphoma cells. (1985) Mutation Research 151:147-159.

OECD Guideline for the Testing of Chemicals, Guideline 476 (*In Vitro* Mammalian Cell Gene Mutation Test), July 1997.

14.0 APPROVAL

Michael F. Girard Sponsor Study Monitor	11/19/98 Date
Michael L. Dourson, Ph.D., DABT Sponsor Scientific Advisor	11-10-98 Date
Richard H.C. San, Ph.D. BioReliance Study Director	12/2/98 Date

If submission to Japanese Regulatory Agency is indicated in section 12.0, BioReliance management will sign.

David Jacobson-Kram, Ph.D., DABT BioReliance Study Management	Date

5.3 GENOTOXICITY ASSAYS

ManTech Environmental Technology, Inc., performed a battery of three genotoxicity assays (*Salmonella typhimurium*/microsome mutagenesis assay [Ames assay], the mouse lymphoma cell mutagenesis assay [L5178Y-TK test], and the in vivo mouse bone marrow micronucleus induction assay) with ammonium perchlorate to help determine its potential for various interactions with DNA and to gain insight on its possible carcinogenicity (ManTech Environmental Technology, Inc., 1998). To confirm the findings of ManTech Environmental Technology, the EPA requested the National Toxicology Program to also evaluate ammonium perchlorate in the Ames assay and the mouse bone marrow micronucleus test (NTP, 1999a). The sponsor (PSG) also had the mouse lymphoma assay repeated (BioReliance, 1999).

Ammonium Perchlorate was evaluated in the Ames assay (*Salmonella typhimurium*/ microsome mutagenesis assay), which is a well-defined assay for detection of carcinogens/ mutagens. It measures the reversion from a his^- (histidine independent) state induced by chemicals that cause base-pair changes or frameshift mutations in the genome of the organism (i.e., it measures for point mutations [e.g., substitution, addition, or deletion of one or a few DNA base pairs within a gene]). In this assay, bacteria are exposed to the test chemical with and without a metabolic activation system (Arochlor 1254-induced rat liver S9 with co-factors). The mutagenicity is evaluated by the increase in the number of revertant colonies. The L5178Y mouse-lymphoma assay is another short term in vitro assay to detect both point mutations and structural chromosomal changes. The in vivo mammalian micronucleus test detects the damage of chromosomes or of the mitotic apparatus caused by a clastogenic chemical in bone marrow cells (polychromatic erythrocyte [PCE] stem cells) of treated animals. Micronuclei are believed to be formed from chromosomes or chromosome fragments left behind during anaphase of mitosis. The induction of micronuclei indicates changes in either chromosome structure or number in bone marrow cells. ManTech Environmental Technology, Inc., performed this assay in Swiss-CD-1 mice and the NTP used B6C3F1 mice (NTP, 1999a). The micronucleus assay also was performed as part of the 90-day bioassay in Spraque-Dawley rats (Springborn Laboratories, Inc., 1998). This is an adequate series of tests to determine the mutagenic and clastogenic (chromosomal breaking) potential of an agent. It should be noted that perchlorate is not likely to be mutagenic, given its physical and chemical properties (i.e., it is simply an anion). Although perchlorate is an oxidizing agent, it is not expected to produce oxidative DNA damage because of the kinetic considerations discussed in Chapter 2.

5.3.1 In Vitro Assays

Ammonium perchlorate was not found to be mutagenic in the *Salmonella typhimurium* (Ames assay) with and without Arochlor 1254-induced rat liver S9 activation by two separate laboratories (ManTech Environmental Technology, Inc., 1998; NTP, 1999b). In the ManTech study, ammonium perchlorate was dissolved in distilled water and tested at five concentrations (5,000, 2,500, 1,250, 625, and 312.5 µg/plate) in tester strains TA98, TA100, TA1535, and TA1537, without and with Arochlor 1254-induced rat liver S9 using the plate incorporation assay. Although this study was regarded as adequate, the EPA requested that Ames assay be repeated by the National Toxicology Program (NTP) to confirm the negative findings and to include additional tester strains (i.e., TA102 and TA104) which are able to detect a variety of oxidative mutagens. Therefore, NTP evaluated ammonium perchlorate in the Salmonella/Ames assay in tester strains TA98, TA100, TA1535, TA97, TA102, and TA104 (NTP, 1999b). Ammonium perchlorate was dissolved in distilled water and tested using the preincubation procedure at doses of 10,000, 3,333, 1,000, 333, and 100 µg/plate, with and without metabolic activation from Arochlor-induced rat and hamster livers. Ammonium perchlorate was neither toxic nor mutagenic under the conditions of the NTP assay.

The L5178Y/$tk^{+/-}$ mouse lymphoma assay also was used to evaluate the mutagenic and chromosomal breaking potential of ammonium perchlorate in vitro. Ammonium perchlorate was reported to be negative both in the absence and presence of rat Arochlor-induced S9 liver activation (ManTech Environmental Technology, Inc., 1998). Ammonium perchlorate was evaluated at 5.0, 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL without S9 activation, and at 2.5, 0.5, 0.25, 0.05, and 0.025 mg/mL with S9 activation. Although a small increase in mutation frequency was found in the absence of S9 activation at 2.5 mg/mL, which appeared to be statistically significant ($p < 0.05$) by the two-tail, Student's t-test, a repeat assay found no increase in mutation frequency at this concentration compared with controls. Therefore, ammonium perchlorate is considered to be negative in the absence of S9 activation. Confidence in the negative findings without S9 activation is reinforced by the wide range of ammonium perchlorate concentrations evaluated. Although ammonium perchlorate also was reported as negative in the presence of S9 activation, the response of the positive control, 3-methyl cholanthrene (MCA), in the actual experiment was too low (182.6×10^{-6}) to be acceptable. The highest dose of ammonium perchlorate produced a mutation frequency of 194×10^{-6}. The MCA at 2.5 μg/mL should induce a mutation frequency of 300 to 350×10^{-6} or higher. Such a low positive control response weakens the confidence for the negative finding with S9 activation. In addition, the cloning efficiencies for the S9 test appear to be too high (143%), further reducing the confidence in a negative finding. Therefore, only the assays on ammonium perchlorate without S9 are considered unequivocally to be negative. Although perchlorate is not expected to be metabolized to a mutagenic intermediate, these S9 data are not of sufficient quality to support a clear negative-response conclusion.

Because of the problems described above, the sponsor (PSG) had the mouse lymphoma assay repeated. In this recent mouse lymphoma assay, ammonium perchlorate was evaluated at concentrations of 1000, 2000, 3000, 4000, and 5000 ug/ml without and with Arochlor 1254-induced rat liver S9 activation (BioReliance, 1999). No increase in mutant frequencies were found after treatment with perchlorate. The data are judged to be of sufficient quality to determine perchlorate to be nonmutagenic both with and without S9 activation. Although the background mutant frequency was low, particularly in the without S9 experiment, the data set still is considered to be overall very good., as well as internally consistent. The problems that were observed in the data generated by the first laboratory (ManTech Environmental Technology, Inc., 1998) are not present in the data from the BioReliance study

5.3.2 In Vivo Assays

The potential for ammonium perchlorate to induce micronuclei was evaluated in mice and rats. Ammonium perchlorate was administered by drinking water gavage for 3 consecutive days to Swiss CD-1 mice (5 females and 5 males per dose group) at 1,000, 500, 250, 125, and

62.5 mg/kg-day (ManTech Environmental Technology, Inc., 1998). Twenty-four hours after the last dose, the mice were sacrificed, and the frequency of micronucleated cells were evaluated by counting 1,000 PCEs per animal. The assay was conducted in accordance with existing EPA FIFRA/TSCA testing guidelines. No increase in the frequency of micronuclei were found for any dose group. There is some uncertainty whether a maximum tolerated dose (MTD) was reached in this study. The study authors reported that at 2,000 mg/kg, 4 out of 6 animals died after one dosing of ammonium perchlorate. Typically, the assay is performed at 85% of the MTD, and the 1,000 mg/kg-day represents approximately 50% of the LD_{50}. There was no indication of toxicity to the bone marrow cells because the PCE/NCE ratio was not different from negative controls. Furthermore, the study authors did not report any indication of clinical signs of toxicity in the highest dose group. Despite a rebuttal submitted by Dourson (1998) on behalf of the sponsor (PSG), EPA remained concerned because of the importance of this test in the overall determination of the approach to be taken for the carcinogenicity assessment (i.e., to rule out direct genotoxicity).

The NTP agreed to expedite and repeat this test in response to an EPA request. The assay was performed by ip injection to ensure the greatest delivery to the bone marrow. Male B6C3F1 mice were treated with 125, 250, 500, 1,000, 1,500, and 2,000 mg/kg ammonium perchlorate in buffered saline, plus solvent and positive (cyclophosphamide) controls. Note that this study uses two dose groups higher than those used in the previous study (i.e., 1,500 and 2,000 mg/kg). Furthermore, use of ip injection as the route of administration would result in a direct delivery of the compound to the bone marrow cells versus drinking water gavage. Five mice per group were injected daily for 3 consecutive days and were sacrificed 24 h after the last injection; 2,000 PCEs were scored per animal for micronuclei. All animals in the 1,500- and 2,000-mg/kg groups died after the first ip injection, and 4/5 animals died in the 1,000-mg/kg group after the second ip injection. No increases in percent PCE were observed in any of the remaining test groups (125, 250, and 500 mg/kg). No bone marrow toxicity was seen as indicated by the percent of PCE. These results are interpreted to be consistent with those of the ManTech Environmental Technology, Inc. (1998) study that used gavage drinking water administration, and confirm that perchlorate does not induce micronuclei in rodents.

The 90-day subchronic bioassay using Spraque-Dawley rats also evaluated micronuclei induction (Springborn Laboratories, Inc., 1998). The frequency of micronuclei induction was examined in both the males and females after the 90-day sacrifice in the 10-mg/kg-day dose group of ammonium perchlorate administered by drinking water. Although there was no induction of micronuclei at this dose, 10 mg/kg-day does not appear to reach a MTD because there were no overt signs of toxicity, although the definition of MTD may be somewhat moot, given the changes in thyroid hormone economy and histopathology seen in the thyroids at that dose. There was significant reduction in the PCE/NCE ratio (i.e, an indicator of toxicity to the bone marrow cells).

5.3.3 Summary of Genotoxicity Battery Results

Negative results were reported in all genotoxicity assays conducted on ammonium perchlorate when evaluated by two independent laboratories. Ammonium perchlorate was not mutagenic in the Ames assay (with or without S9 activation). Negative results were also found in the mouse lymphoma gene mutation assay without and with S9 activation. Ammonium perchlorate did not induce chromosomal anomalies when evaluated for micronuclei induction in the bone marrow of mice when administered via drinking water gavage or i.p. injection No increases in micronuclei were found in Spraque-Dawley rats when evaluated from the 90-day study at the highest dose, which produced both thyroid hormone perturbations and follicular cell hyperplasia. It is concluded that ammonium perchlorate does not have the potential to be mutagenic or clastogenic. The in vitro and in vivo studies discussed above provide support for that conclusion. Therefore, mutagenicity is not considered a possible mode of carcinogenic action for this chemical.

February 1, 1999 EPA Assessment Submission

Attachment #2
Analysis of Brain Histopathology at 3 mg/kg-day
Argus (1998a) Neurodevelopmental Study

A. Argus 1/20/98 Data Submission (York, 1998f)
B. EPA analysis (Geller, 1999a)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

November 20, 1998

Annie Jarabek
USEPA, National Center for
Environmental Assessment
3210 Highway 54, Catawba Bldg.
Research Triangle Park, NC 27709

Telephone: (919) 541-4847
Telefax: (919) 541-1818

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Ms. Jarabek:

Enclosed is a diskette containing the thyroid hormone data for the Fo generation adults and F1 generation pups supplied by AniLytics, as well as a summary table created by Argus to show the mean group values and identify which groups are significantly different than control values. Please note that there is an error in the data supplied by AniLytics. For the F1 generation females, pup number 3668 has been incorrectly identified as being in the 30.0 mg/kg/day dosage group, and should be 3.0 mg/kg/day. The summary table does reflect this correction. AniLytics has been made aware of this incorrect value and will make the necessary changes to their data.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Ray

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:hmg
Enc.
Copies to: D. Mattie
M. Dourson

Protocol 1416-001: Summary of Thyroid Hormone Data

Fo Generation Rats:

Dosage Group	Dosage Level (mg/kg/day)	TSH (ng/mL)		T3 (ng/dL)		T4 (µg/dL)	
		Male Rats	Female Rats	Male Rats	Female Rats	Male Rats	Female Rats
I	0	1.530	2.054	72.547	57.770	4.641	2.126
II	0.3	1.353	2.213	87.389**	64.789	4.726	2.903**
III	3.0	1.487	1.990	88.452**	56.350	4.744	2.924**
IV	30	3.871**	2.174	78.570	60.373	3.578**	2.421

F1 Generation Pups:

Dosage Group	Dosage Level (mg/kg/day)	TSH (ng/mL)		T3 (ng/dL)		T4 (µg/dL)	
		Male Pups	Female Pups	Male Pups	Female Pups	Male Pups	Female Pups
I	0	1.237	1.120	105.897	105.954	4.403	4.270
II	0.3	.941**	1.188	111.150	109.922	4.615	4.865*
III	3.0	.877**	1.141	109.810	109.293	4.533	4.324
IV	30	1.270	1.301	107.398	97.581*	4.525	3.913

* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 27 January 1999

Subject: Analysis of the Brain Morphometry Data from the Neurobehavioral Developmental Study of Ammonium Perchlorate (Argus, 1998a)

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is the statistical analysis of the hormone data from the Argus Neurobehavioral Developmental Study (Argus Protocol #1613-002). Data was received from Argus on November 5, 1998 (York, 1998d) and imported in ASCII form to SAS for further analysis. I have attached a description of how the analyses were done, a description of results, and summary graphs.

Analyses of Brain Morphometry Data from Neurobehavioral Developmental Study (Argus, 1998a)

Summary: A memo from Argus Laboratories (York, 1998d) contains brain morphometry data from the control, 3 mg/kg/day and 10 mg/kg/day dose groups from the Neurobehavioral Developmental Study of ammonium perchlorate in the rat at post-natal day 12 in the F1 generation (Argus, 1998a). This memo adds the morphometric data from the 3 mg/kg/day data to that of the control and high dose (10 mg/kg/day) groups previously reported in Tables 1 and 2 of Appendix P (Argus, 1998a). This data had been requested by the USEPA after initial findings of a morphometric increase in the size of the corpus callosum in the high dose group relative to controls. At the time that the report on Perchlorate Environmental Contamination had been prepared for External Review, only the data from the corpus callosum had been re-analyzed by the USEPA (Crofton, 1998c). The results of analysis of the morphometry data from the other brain regions is reported here..

Data was analyzed using a 2-way analysis of variance, with dose and sex as independent variables. It is desirable in the analysis of developmental data to have litter information; since none was included in Appendix P (Argus, 1998a) or the memo (York, 1998d), it is possible that the effects of sex and litter are confounded.

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen.

The corpus callosum showed an increase in size at the highest dose tested (10 mg/kg /day). The other significant dose effects were driven by effects at the 3.0 mg/kg/day dose group. There was a significant decrease in size in this dose group in hippocampal gyrus and caudate putamen and a significant increase in size in anterior/posterior cerebellum.

Data: All data were supplied in the form of a memo (York, 1998d). Data were keyed in and entered as ASCII files for analyses by SAS.

Data for dependent measures (brain weight, anterior/posterior cerebrum, anterior/posterior cerebellum, frontal cortex, parietal cortex, caudate putamen, corpus callosum, hippocampal gyrus, cerebellum, external germinal layer) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

To correct for multiple comparisons the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.016 (alpha of 0.05 divided by the square root of the number of ANOVAs).

Analyses of Brain Morphometry Data from Neurobehavioral Developmental Study (Argus, 1998a)

Summary: A memo from Argus Laboratories (York, 1998d) contains brain morphometry data from the control, 3 mg/kg/day and 10 mg/kg/day dose groups from the Neurobehavioral Developmental Study of ammonium perchlorate in the rat at post-natal day 12 in the F1 generation (Argus, 1998a). This memo adds the morphometric data from the 3 mg/kg/day data to that of the control and high dose (10 mg/kg/day) groups previously reported in Tables 1 and 2 of Appendix P (Argus, 1998a). This data had been requested by the USEPA after initial findings of a morphometric increase in the size of the corpus callosum in the high dose group relative to controls. At the time that the report on Perchlorate Environmental Contamination had been prepared for External Review, only the data from the corpus callosum had been re-analyzed by the USEPA (Crofton, 1998c). The results of analysis of the morphometry data from the other brain regions is reported here.

Data was analyzed using a 2-way analysis of variance, with dose and sex as independent variables. It is desirable in the analysis of developmental data to have litter information; since none was included in Appendix P (Argus, 1998a) or the memo (York, 1998d), it is possible that the effects of sex and litter are confounded.

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen. An effect of sex was also found in caudate putamen.

The corpus callosum showed an increase in size at the highest dose tested (10 mg/kg /day). The other significant dose effects were driven by effects at the 3.0 mg/kg/day dose group. There was a significant decrease in size in this dose group in hippocampal gyrus and caudate putamen and a significant increase in size in anterior/posterior cerebellum.

Data: All data were supplied in the form of a memo (York, 1998d). Data were keyed in and entered as ASCII files for analyses by SAS.

Data for dependent measures (brain weight, anterior/posterior cerebrum, anterior/posterior cerebellum, frontal cortex, parietal cortex, caudate putamen, corpus callosum, hippocampal gyrus, cerebellum, external germinal layer) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

To correct for multiple comparisons the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.016 (alpha of 0.05 divided by the square root of the number of ANOVAs).

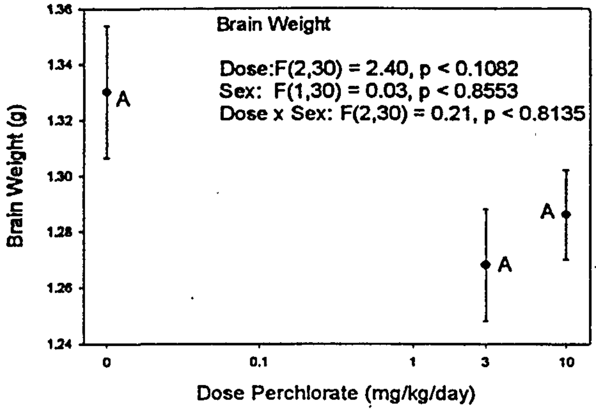
Data Analysis - Results:

Significant effects of dose were found in corpus callosum, hippocampal gyrus, anterior/posterior cerebellum, and caudate putamen (Figure 1). An effect of sex was also found in caudate putamen.

Corpus callosum showed an increase in size in the 10 mg/kg/day dose group, as previously reported in Crofton (1998c).

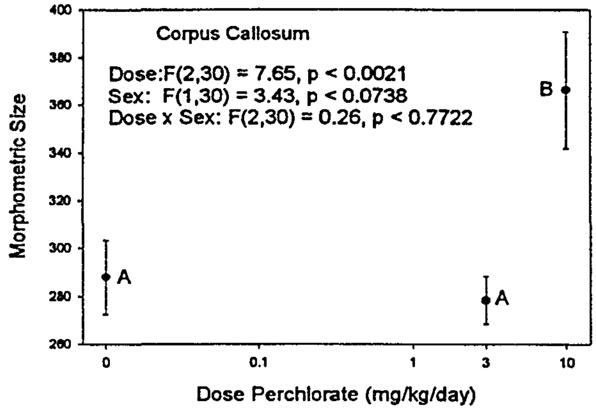
Hippocampal gyrus (12% less than control) and caudate putamen (7.3% less than control) showed a decrease in size at the 3 mg/kg/day dose, with no significant difference between control and high dose, yielding a U-shaped dose response. A/P cerebellum showed a significant increase in size in the 3 mg/kg/day group (13% greater than control), yielding an inverted U-shaped dose response function.

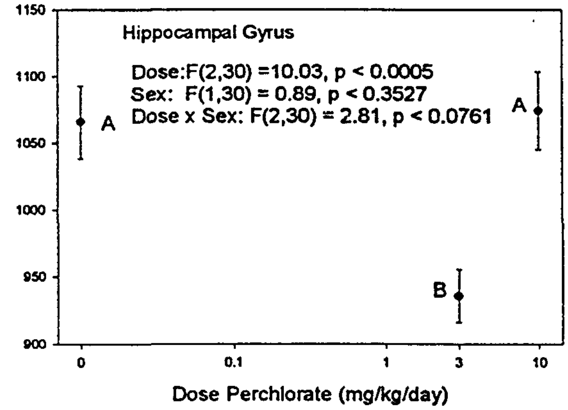
Inhibition of iodide uptake is highly non-linear and saturable, and therefore does not rule out the possibility of a U-shaped dose response. Until the PBPK modeling better characterizes this phenomenon, we are not requesting histopathological evaluation of brain sections at the next lower dose. This is pending commentary with respect to the potential for U-shaped dose response for changes in brain morphology with perchlorate exposure and other recommendations made at the external peer review. We do request, however, that the tissue samples be saved until a final decision is made on this matter.

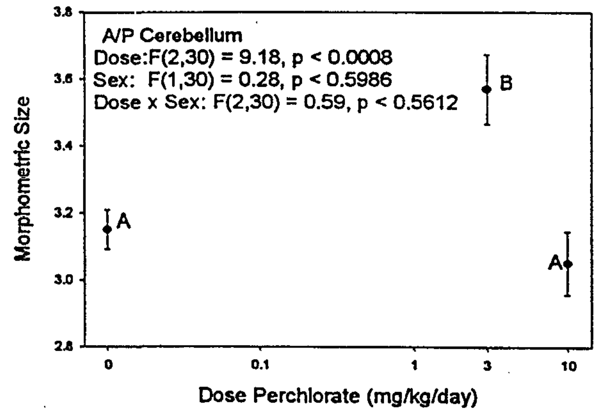


Neurobehavioral Developmental Study of Ammonium Perchlorate in Drinking Water (Argus, 1998a)

F1 Generation, PND12, Male and Female Combined. Brain weight and morphometric size measurements for different brain regions. There is no effect on brain weight. Other plots show regions where significant effect of dose was found in 2-way analysis of variance (independent variables = dose, sex). Within each plot, means with different letters are significantly different ($p < 0.05$).







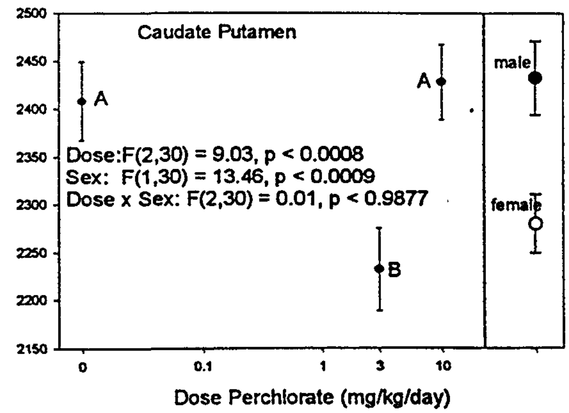


Figure 1

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NOTE: SAS (r) Proprietary Software Release 6.12  TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.


WARNING: Your system is scheduled to expire on February 18, 1999, which is 23 days from now. Please contact your installation
         representative to have your system renewed. The SAS system will no longer function on or after that date.
 Welcome to the NHEERL-RTP SAS Information Delivery System.


          *THIS FILE IS FOUND AT [CRofton.THYROID.perchlorate]perchlorate_dn_pnd5.SAS;
          *IT ANALYZES THE THYROID HORMONE DATA FROM THE WPAFB 90 DAY PERCHLORATE STUDY;


          *INPUT DATA INTO SAS DATASET;
          DATA RAW; INFILE '[GELLER.BMD]1613-002.Txt';
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
               INPUT SEX$ DOSE$ RATNO BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX
                        CAUDPUT CORPCOL HIPPO CEREBLL XGEM;

          * BRAINWT = TOTAL BRAIN WEIGHT;
          * CEREBRUM = ANTER/POST CEREBRUM;
          * APCBLM = ANT/POST CEREBELLUM;
          * FCORTEX = FRONTAL CORTEX;
          * PCORTEX = PARIETAL CORTEX;
          * CAUDPUT = CAUDATE PUTAMEN;
          * CORPCOL = CORPUS CALLOSUM;
          * HIPPO = HIPPOCAMPAL GYRUS;
          * CEREBLL = CEREBELLUM;
          * XGEM = EXT GERM LAYER;

          *ASSIGN TREATMENT VALUES TO DOSE CODES;
               IF DOSE = '1' THEN TRT = '1--------CONTROL';
               IF DOSE = '2' THEN TRT = '2--0.1_mg/kg/day';
               IF DOSE = '3' THEN TRT = '3--1.0_mg/kg/day';
               IF DOSE = '4' THEN TRT = '4--3.0_mg/kg/day';
               IF DOSE = '5' THEN TRT = '5-10.0_mg/kg/day';


NOTE: The infile '[GELLER.BMD]1613-002.Txt' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]1613-002.TXT

NOTE: 36 records were read from the infile '[GELLER.BMD]1613-002.Txt'.
      The minimum record length was 73.
      The maximum record length was 73.
NOTE: The data set WORK.RAW has 36 observations and 14 variables.

                PROC PRINT;
```

```
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
29                    TITLE "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
30
31          *SORT DATA BY TRT -- THEN GET MEANS;
32
33
12                                                     The SAS System                      15:56 Tuesday, January 26, 1999


NOTE: The PROCEDURE PRINT printed page 1.

33           PROC SORT; BY TRT;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
34

NOTE: The data set WORK.RAW has 36 observations and 14 variables.

34           PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY TRT;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
35                    VAR BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
36                            CORPCOL HIPPO CEREBLL XGEM;;
37                    TITLE1 "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
38                    TITLE2 "GROUP MEANS BY TREATMENT";
39
40          *SORT DATA BY TRT AND GENDER -- THEN GET MEANS;
41
42

NOTE: The PROCEDURE MEANS printed page 2.

42           PROC SORT; BY TRT SEX;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
43

NOTE: The data set WORK.RAW has 36 observations and 14 variables.

43           PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY TRT SEX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
44                    VAR BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
45                            CORPCOL HIPPO CEREBLL XGEM;
46                    TITLE1 "PERCHLORATE NEURODEVELOPMENTAL ARGUS 1613-002 - RAW DATA";
47                    TITLE2 "GROUP MEANS BY GENDER AND TREATMENT";
48
49          *RUN ONE WAY ANOVAs FOR ALL VARIABLES;
50

NOTE: The PROCEDURE MEANS printed pages 3-4.
```

```
        PROC SORT; BY TRT SEX;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.

NOTE: Input data set is already sorted, no sorting done.

        PROC GLM;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
                 CLASSES TRT SEX;
                 MODEL BRAINWT CEREBRUM APCBLM FCORTEX PCORTEX CAUDPUT
                        CORPCOL HIPPO CEREBLL XGEM = TRT|SEX;
                 MEANS TRT/TUKEY LINES;
13                                        The SAS System                    15:56 Tuesday, January 26, 1999

                 TITLE1 "ARGUS DEVELOPMENTAL NEURO PND12 CNS MORPHOMETRICS";
                      TITLE2 "PROC GLM - WITH TUKEYS";
       ENDSAS;

NOTE: Means from the MEANS statement are not adjusted for other terms in the model.  For adjusted means, use the LSMEANS statement.
NOTE: The PROCEDURE GLM printed pages 5-25.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

OBS	SEX	DOSE	RATNO	BRAINWT	CEREBRUM	APCBLM	FCORTEX	PCORTEX	CAUDPUT	CORPCOL	HIPPO	CEREBLL	XGEM	TRT
1	F	1	2122	1.233	12.6	3.0	1224	1344	2208	192	912	3120	43	1--------CONTROL
2	F	1	2136	1.365	12.8	3.5	1512	1440	2448	259	1056	3696	36	1--------CONTROL
3	F	1	2170	1.342	12.9	3.0	1584	1512	2304	288	1104	3600	41	1--------CONTROL
4	F	1	2172	1.517	13.5	3.0	1632	1536	2496	298	1128	3984	41	1--------CONTROL
5	F	1	2185	1.321	12.5	3.2	1416	1296	2208	269	1152	3552	48	1--------CONTROL
6	F	1	2194	1.280	12.5	2.9	1536	1488	2304	336	960	3552	41	1--------CONTROL
7	F	2	2132	1.259	12.6	4.0	1440	1392	2304	259	984	3360	48	2--0.1_mg/kg/day
8	F	2	2133	1.168	12.3	3.7	1440	1392	2160	269	840	3072	46	2--0.1_mg/kg/day
9	F	2	2145	1.419	13.2	3.3	1560	1656	2256	288	1008	3840	41	2--0.1_mg/kg/day
10	F	2	2151	1.212	12.8	3.5	1488	1416	2016	269	1080	3456	41	2--0.1_mg/kg/day
11	F	2	2165	1.222	12.5	3.3	1488	1488	2064	259	912	3360	41	2--0.1_mg/kg/day
12	F	2	2174	1.347	13.2	4.1	1440	1392	2160	250	960	3696	43	2--0.1_mg/kg/day
13	F	3	2123	1.278	12.4	3.4	1344	1392	2304	307	1080	3024	41	3--1.0_mg/kg/day
14	F	3	2124	1.310	12.9	3.0	1296	1440	2400	336	1032	3552	36	3--1.0_mg/kg/day
15	F	3	2140	1.182	12.6	3.0	1464	1464	2352	355	1056	3264	36	3--1.0_mg/kg/day
16	F	3	2143	1.254	12.9	3.0	2198	1440	2448	346	1008	3168	36	3--1.0_mg/kg/day
17	F	3	2193	1.314	12.6	2.9	1392	1512	2256	355	936	3696	41	3--1.0_mg/kg/day
18	F	3	2198	1.330	13.2	3.3	1632	1608	2352	326	1008	3504	41	3--1.0_mg/kg/day
19	M	1	2002	1.375	13.2	3.4	1440	1416	2592	278	1080	3888	41	1--------CONTROL
20	M	1	2008	1.213	12.7	3.2	1296	1344	2400	240	1056	3648	36	1--------CONTROL
21	M	1	2036	1.357	12.7	3.2	1224	1368	2640	336	1248	3552	36	1--------CONTROL
22	M	1	2062	1.252	12.5	2.9	1368	1368	2352	240	936	3168	41	1--------CONTROL
23	M	1	2067	1.389	13.0	3.4	1368	1392	2544	384	1080	3696	41	1--------CONTROL
24	M	1	2094	1.335	13.2	3.1	1560	1632	2400	336	1080	3216	36	1--------CONTROL
25	M	2	2001	1.335	13.0	3.5	1464	1440	2400	365	984	3456	41	2--0.1_mg/kg/day
26	M	2	2019	1.289	13.0	3.5	1440	1440	2496	307	912	3312	36	2--0.1_mg/kg/day
27	M	2	2026	1.240	13.1	3.1	1392	1368	2304	259	888	3360	34	2--0.1_mg/kg/day
28	M	2	2039	1.250	13.1	3.8	1512	1488	2304	307	912	3312	31	2--0.1_mg/kg/day
29	M	2	2076	1.267	12.6	4.0	1272	1464	2016	240	864	3216	24	2--0.1_mg/kg/day
30	M	2	2097	1.208	12.3	3.0	1464	1464	2304	269	888	3264	43	2--0.1_mg/kg/day
31	M	3	2010	1.356	13.0	3.2	1608	1584	2640	528	1152	3504	36	3--1.0_mg/kg/day
32	M	3	2020	1.194	13.0	3.0	1584	1464	2688	317	984	3168	41	3--1.0_mg/kg/day
33	M	3	2028	1.249	12.7	2.2	1080	1296	2544	557	1200	3120	36	3--1.0_mg/kg/day
34	M	3	2037	1.353	13.0	3.5	1344	1512	2400	307	1032	3792	36	3--1.0_mg/kg/day
35	M	3	2041	1.289	13.0	3.2	1080	1440	2304	298	1104	3216	41	3--1.0_mg/kg/day
36	M	3	2043	1.321	13.0	2.9	1080	1488	2448	365	1296	3744	41	3--1.0_mg/kg/day

GROUP MEANS BY TREATMENT

------------ TRT=1--------CONTROL ------------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.3315833	0.0237300	1.2130000	1.5170000	0.0822031	0.0067574	6.1733379
CEREBRUM	12	12.8416667	0.0941134	12.5000000	13.5000000	0.3260182	0.1062879	2.5387532
APCBLM	12	3.1500000	0.0583874	2.9000000	3.5000000	0.2022600	0.0409091	6.4209511
FCORTEX	12	1430.00	39.9044313	1224.00	1632.00	138.2330049	19108.36	9.6666437
PCORTEX	12	1428.00	28.1037041	1296.00	1632.00	97.3540866	9477.82	6.8175131
CAUDPUT	12	2408.00	40.8634088	2208.00	2640.00	141.5550006	20037.82	5.8785299
CORPCOL	12	288.0000000	15.4120181	192.0000000	384.0000000	53.3887969	2850.36	18.5377767
HIPPO	12	1066.00	27.3096719	912.0000000	1248.00	94.6034787	8949.82	8.8746228
CEREBLL	12	3556.00	77.8156329	3120.00	3984.00	269.5612597	72663.27	7.5804629
XGEM	12	40.0833333	1.0405297	36.0000000	48.0000000	3.6045006	12.9924242	8.9925170

------------ TRT=2--0.1_mg/kg/day ------------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.2680000	0.0202286	1.1680000	1.4190000	0.0700740	0.0049104	5.5263397
CEREBRUM	12	12.8083333	0.0972799	12.3000000	13.2000000	0.3369875	0.1135606	2.6310023
APCBLM	12	3.5666667	0.1039619	3.0000000	4.1000000	0.3601347	0.1296970	10.0972333
FCORTEX	12	1450.00	20.3514574	1272.00	1560.00	70.4995164	4970.18	4.8620356
PCORTEX	12	1450.00	22.0000000	1368.00	1656.00	76.2102355	5808.00	5.2558783
CAUDPUT	12	2232.00	43.6181780	2016.00	2496.00	151.0978010	22830.55	6.7696147
CORPCOL	12	278.4166667	9.8868280	240.0000000	365.0000000	34.2489770	1172.99	12.3013386
HIPPO	12	936.0000000	19.8173478	840.0000000	1080.00	68.6493064	4712.73	7.3343276
CEREBLL	12	3392.00	59.4765041	3072.00	3840.00	206.0326541	42449.45	6.0740759
XGEM	12	39.0833333	1.9480306	24.0000000	48.0000000	6.7481760	45.5378788	17.2661219

------------ TRT=3--1.0_mg/kg/day ------------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	12	1.2858333	0.0164353	1.1820000	1.3560000	0.0569335	0.0032414	4.4277517
CEREBRUM	12	12.8583333	0.0668086	12.4000000	13.2000000	0.2314316	0.0535606	1.7998572
APCBLM	12	3.0500000	0.0957427	2.2000000	3.5000000	0.3316625	0.1100000	10.8741796
FCORTEX	12	1425.17	90.8126471	1080.00	2198.00	314.5842374	98963.24	22.0735051
PCORTEX	12	1470.00	23.8403783	1296.00	1608.00	82.5854929	6820.36	5.6180607
CAUDPUT	12	2428.00	38.9498512	2256.00	2688.00	134.9262425	18205.09	5.5570940
CORPCOL	12	366.4166667	24.5956569	298.0000000	557.0000000	85.2018548	7259.36	23.2527236
HIPPO	12	1074.00	29.1141952	936.0000000	1296.00	100.8545307	10171.64	9.3905522
CEREBLL	12	3396.00	77.0643179	3024.00	3792.00	266.9586281	71266.91	7.8609726
XGEM	12	38.5000000	0.7537784	36.0000000	41.0000000	2.6111648	6.8181818	6.7822463

GROUP MEANS BY GENDER AND TREATMENT

------------ TRT=1--------CONTROL SEX=F ------------

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.3430000	0.0397131	1.2330000	1.5170000	0.0972769	0.0094628	7.2432557
CEREBRUM	6	12.8000000	0.1549193	12.5000000	13.5000000	0.3794733	0.1440000	2.9646353
APCBLM	6	3.1000000	0.0894427	2.9000000	3.5000000	0.2190890	0.0480000	7.0673878
FCORTEX	6	1484.00	59.8932383	1224.00	1632.00	146.7078730	21523.20	9.8859753
PCORTEX	6	1436.00	39.3954312	1296.00	1536.00	96.4987047	9312.00	6.7199655
CAUDPUT	6	2328.00	49.1853637	2208.00	2496.00	120.4790438	14515.20	5.1752167
CORPCOL	6	273.6666667	19.6547987	192.0000000	336.0000000	48.1442278	2317.87	17.5922879
HIPPO	6	1052.00	39.3954312	912.0000000	1152.00	96.4987047	9312.00	9.1728807
CEREBLL	6	3584.00	114.0385900	3120.00	3984.00	279.3363564	78028.80	7.7939832
XGEM	6	41.6666667	1.5846486	36.0000000	48.0000000	3.8815804	15.0666667	9.3157930

--- TRT=1--------CONTROL SEX=M ---

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.3201667	0.0291278	1.2130000	1.3890000	0.0713482	0.0050906	5.4044848
CEREBRUM	6	12.8833333	0.1194897	12.5000000	13.2000000	0.2926887	0.0856667	2.2718397
APCBLM	6	3.2000000	0.0774597	2.9000000	3.4000000	0.1897367	0.0360000	5.9292706
FCORTEX	6	1376.00	47.4636703	1224.00	1560.00	116.2617736	13516.80	8.4492568
PCORTEX	6	1420.00	43.5614508	1344.00	1632.00	106.7033270	11385.60	7.5143188
CAUDPUT	6	2488.00	48.6621002	2352.00	2640.00	119.1973154	14208.00	4.7908889
CORPCOL	6	302.3333333	24.0134222	240.0000000	384.0000000	58.8206313	3459.87	19.4555561
HIPPO	6	1080.00	40.6349603	936.0000000	1248.00	99.5349185	9907.20	9.2161962
CEREBLL	6	3528.00	115.4330975	3168.00	3888.00	282.7521883	79948.80	8.0145178
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

--- TRT=2--0.1_mg/kg/day SEX=F ---

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2711667	0.0384339	1.1680000	1.4190000	0.0941433	0.0088630	7.4060572
CEREBRUM	6	12.7666667	0.1520234	12.3000000	13.2000000	0.3723797	0.1386667	2.9168125
APCBLM	6	3.6500000	0.1408309	3.3000000	4.1000000	0.3449638	0.1190000	9.4510621
FCORTEX	6	1476.00	19.3494186	1440.00	1560.00	47.3962024	2246.40	3.2111248
PCORTEX	6	1456.00	42.7831743	1392.00	1656.00	104.7969465	10982.40	7.1975925
CAUDPUT	6	2160.00	44.6855681	2016.00	2304.00	109.4568408	11980.80	5.0674463
CORPCOL	6	265.6666667	5.3395797	250.0000000	288.0000000	13.0792456	171.0666667	4.9231790
HIPPO	6	964.0000000	33.6095225	840.0000000	1080.00	82.3261805	6777.60	8.5400602
CEREBLL	6	3464.00	111.1395519	3072.00	3840.00	272.2351924	74112.00	7.8589836
XGEM	6	43.3333333	1.2292726	41.0000000	48.0000000	3.0110906	9.0666667	6.9486706

GROUP MEANS BY GENDER AND TREATMENT

--- TRT=2--0.1_mg/kg/day SEX=M ---

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2648333	0.0178688	1.2080000	1.3350000	0.0437695	0.0019158	3.4604932
CEREBRUM	6	12.8500000	0.1335415	12.3000000	13.1000000	0.3271085	0.1070000	2.5455918
APCBLM	6	3.4833333	0.1579381	3.0000000	4.0000000	0.3868678	0.1496667	11.1062516
FCORTEX	6	1424.00	34.3161769	1272.00	1512.00	84.0571234	7065.60	5.9028879
PCORTEX	6	1444.00	16.8760185	1368.00	1488.00	41.3376342	1708.80	2.8627170
CAUDPUT	6	2304.00	65.5804849	2016.00	2496.00	160.6387251	25804.80	6.9721669
CORPCOL	6	291.1666667	18.3456020	240.0000000	365.0000000	44.9373638	2019.37	15.4335537
HIPPO	6	908.0000000	16.8760185	864.0000000	984.0000000	41.3376342	1708.80	4.5526029
CEREBLL	6	3320.00	33.7520370	3216.00	3456.00	82.6752684	6835.20	2.4902189
XGEM	6	34.8333333	2.8215441	24.0000000	43.0000000	6.9113433	47.7666667	19.8411770

TRT=3--1.0_mg/kg/day SEX=F

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2780000	0.0222231	1.1820000	1.3300000	0.0544353	0.0029632	4.2594118
CEREBRUM	6	12.7666667	0.1173788	12.4000000	13.2000000	0.2875181	0.0826667	2.2521001
APCBLM	6	3.1000000	0.0816497	2.9000000	3.4000000	0.2000000	0.0400000	6.4516129
FCORTEX	6	1554.33	137.3350324	1296.00	2198.00	336.4007531	113165.47	21.6427677
PCORTEX	6	1476.00	30.8285582	1392.00	1608.00	75.5142371	5702.40	5.1161407
CAUDPUT	6	2352.00	27.7128129	2256.00	2448.00	67.8822510	4608.00	2.8861501
CORPCOL	6	337.5000000	7.6365350	307.0000000	355.0000000	18.7056141	349.9000000	5.5424042
HIPPO	6	1020.00	20.3174802	936.0000000	1080.00	49.7674592	2476.80	4.8791627
CEREBLL	6	3368.00	104.7358582	3024.00	3696.00	256.5494104	65817.60	7.6172628
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

TRT=3--1.0_mg/kg/day SEX=M

Variable	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance	CV
BRAINWT	6	1.2936667	0.0258865	1.1940000	1.3560000	0.0634087	0.0040207	4.9014734
CEREBRUM	6	12.9500000	0.0500000	12.7000000	13.0000000	0.1224745	0.0150000	0.9457489
APCBLM	6	3.0000000	0.1807392	2.2000000	3.5000000	0.4427189	0.1960000	14.7572957
FCORTEX	6	1296.00	103.6918512	1080.00	1608.00	253.9921259	64512.00	19.5981579
PCORTEX	6	1464.00	39.1918359	1296.00	1584.00	96.0000000	9216.00	6.5573770
CAUDPUT	6	2504.00	59.9733274	2304.00	2688.00	146.9040503	21580.80	5.8667752
CORPCOL	6	395.3333333	47.6337882	298.0000000	557.0000000	116.6784756	13613.87	29.5139483
HIPPO	6	1128.00	46.3724056	984.0000000	1296.00	113.5887318	12902.40	10.0699230
CEREBLL	6	3424.00	121.8523697	3120.00	3792.00	298.4761297	89088.00	8.7171767
XGEM	6	38.5000000	1.1180340	36.0000000	41.0000000	2.7386128	7.5000000	7.1132800

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	3	1--------CONTROL 2--0.1_mg/kg/day 3--1.0_mg/kg/day
SEX	2	F M

General Linear Models Procedure

Dependent Variable: BRAINWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.02823647	0.00564729	1.05	0.4079
Error	30	0.16157983	0.00538599		
Corrected Total	35	0.18981631			

R-Square	C.V.	Root MSE	BRAINWT Mean
0.148757	5.666522	0.07338933	1.29513889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	0.02581572	0.01290786	2.40	0.1082
SEX	1	0.00018225	0.00018225	0.03	0.8553
TRT*SEX	2	0.00223850	0.00111925	0.21	0.8135

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.02581572	0.01290786	2.40	0.1082
SEX	1	0.00018225	0.00018225	0.03	0.8553
TRT*SEX	2	0.00223850	0.00111925	0.21	0.8135

General Linear Models Procedure

Dependent Variable: CEREBRUM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.15805556	0.03161111	0.33	0.8902
Error	30	2.86500000	0.09550000		
Corrected Total	35	3.02305556			

R-Square	C.V.	Root MSE	CEREBRUM Mean
0.052283	2.407511	0.30903074	12.83611111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	0.01555556	0.00777778	0.08	0.9220
SEX	1	0.12250000	0.12250000	1.28	0.2664
TRT*SEX	2	0.02000000	0.01000000	0.10	0.9009

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	0.01555556	0.00777778	0.08	0.9220
SEX	1	0.12250000	0.12250000	1.28	0.2664
TRT*SEX	2	0.02000000	0.01000000	0.10	0.9009

General Linear Models Procedure

Dependent Variable: APCBLM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.94555556	0.38911111	3.97	0.0070
Error	30	2.94333333	0.09811111		
Corrected Total	35	4.88888889			

R-Square	C.V.	Root MSE	APCBLM Mean
0.397955	9.621305	0.31322693	3.25555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	1.80222222	0.90111111	9.18	0.0008
SEX	1	0.02777778	0.02777778	0.28	0.5986
TRT*SEX	2	0.11555556	0.05777778	0.59	0.5612

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	1.80222222	0.90111111	9.18	0.0008
SEX	1	0.02777778	0.02777778	0.28	0.5986
TRT*SEX	2	0.11555556	0.05777778	0.59	0.5612

General Linear Models Procedure

Dependent Variable: FCORTEX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	247472.55555554	49494.51111111	1.34	0.2756
Error	30	1110147.33333334	37004.91111111		
Corrected Total	35	1357619.88888888			

R-Square	C.V.	Root MSE	FCORTEX Mean
0.182284	13.40482	192.36660602	1435.05555556

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	4160.22222222	2080.11111111	0.06	0.9454
SEX	1	175002.77777778	175002.77777778	4.73	0.0377
TRT*SEX	2	68309.55555556	34154.77777778	0.92	0.4083

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	4160.22222222	2080.11111111	0.06	0.9454
SEX	1	175002.77777778	175002.77777778	4.73	0.0377
TRT*SEX	2	68309.55555556	34154.77777778	0.92	0.4083

General Linear Models Procedure

Dependent Variable: PCORTEX

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12224.00000000	2444.80000000	0.30	0.9068
Error	30	241536.00000000	8051.20000000		
Corrected Total	35	253760.00000000			

R-Square	C.V.	Root MSE	PCORTEX Mean
0.048172	6.191017	89.72847931	1449.33333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	10592.00000000	5296.00000000	0.66	0.5253
SEX	1	1600.00000000	1600.00000000	0.20	0.6590
TRT*SEX	2	32.00000000	16.00000000	0.00	0.9980

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	10592.00000000	5296.00000000	0.66	0.5253
SEX	1	1600.00000000	1600.00000000	0.20	0.6590
TRT*SEX	2	32.00000000	16.00000000	0.00	0.9980

General Linear Models Procedure

Dependent Variable: CAUDPUT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	487488.00000000	97497.60000000	6.31	0.0004
Error	30	463488.00000000	15449.60000000		
Corrected Total	35	950976.00000000			

R-Square	C.V.	Root MSE	CAUDPUT Mean
0.512619	5.275739	124.29641990	2356.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	279168.00000000	139584.00000000	9.03	0.0008
SEX	1	207936.00000000	207936.00000000	13.46	0.0009
TRT*SEX	2	384.00000000	192.00000000	0.01	0.9877

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	279168.00000000	139584.00000000	9.03	0.0008
SEX	1	207936.00000000	207936.00000000	13.46	0.0009
TRT*SEX	2	384.00000000	192.00000000	0.01	0.9877

General Linear Models Procedure

Dependent Variable: CORPCOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	70390.22222222	14078.04444444	3.85	0.0082
Error	30	109659.66666667	3655.32222222		
Corrected Total	35	180049.88888889			

R-Square	C.V.	Root MSE	CORPCOL Mean
0.390948	19.44375	60.45926085	310.94444444

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	55940.05555556	27970.02777778	7.65	0.0021
SEX	1	12544.00000000	12544.00000000	3.43	0.0738
TRT*SEX	2	1906.16666667	953.08333333	0.26	0.7722

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	55940.05555556	27970.02777778	7.65	0.0021
SEX	1	12544.00000000	12544.00000000	3.43	0.0738
TRT*SEX	2	1906.16666667	953.08333333	0.26	0.7722

General Linear Models Procedure

Dependent Variable: HIPPO

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	190784.00000000	38156.80000000	5.31	0.0013
Error	30	215424.00000000	7180.80000000		
Corrected Total	35	406208.00000000			

R-Square	C.V.	Root MSE	HIPPO Mean
0.469671	8.264590	84.73960113	1025.33333333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	144032.00000000	72016.00000000	10.03	0.0005
SEX	1	6400.00000000	6400.00000000	0.89	0.3527
TRT*SEX	2	40352.00000000	20176.00000000	2.81	0.0761

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	144032.00000000	72016.00000000	10.03	0.0005
SEX	1	6400.00000000	6400.00000000	0.89	0.3527
TRT*SEX	2	40352.00000000	20176.00000000	2.81	0.0761

General Linear Models Procedure

Dependent Variable: CEREBLL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	291072.00000000	58214.40000000	0.89	0.5021
Error	30	1969152.00000000	65638.40000000		
Corrected Total	35	2260224.00000000			

R-Square	C.V.	Root MSE	CEREBLL Mean
0.128780	7.430392	256.19992194	3448.00000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	210048.00000000	105024.00000000	1.60	0.2186
SEX	1	20736.00000000	20736.00000000	0.32	0.5783
TRT*SEX	2	60288.00000000	30144.00000000	0.46	0.6361

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	210048.00000000	105024.00000000	1.60	0.2186
SEX	1	20736.00000000	20736.00000000	0.32	0.5783
TRT*SEX	2	60288.00000000	30144.00000000	0.46	0.6361

General Linear Models Procedure

Dependent Variable: XGEM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	262.22222222	52.44444444	3.33	0.0164
Error	30	472.00000000	15.73333333		
Corrected Total	35	734.22222222			

R-Square	C.V.	Root MSE	XGEM Mean
0.357143	10.11296	3.96652661	39.22222222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	2	15.38888889	7.69444444	0.49	0.6180
SEX	1	136.11111111	136.11111111	8.65	0.0062
TRT*SEX	2	110.72222222	55.36111111	3.52	0.0424

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	2	15.38888889	7.69444444	0.49	0.6180
SEX	1	136.11111111	136.11111111	8.65	0.0062
TRT*SEX	2	110.72222222	55.36111111	3.52	0.0424

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: BRAINWT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.005386
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.0739

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1.33158	12	1--------CONTROL
A			
A	1.28583	12	3--1.0_mg/kg/day
A			
A	1.26800	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CEREBRUM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.0955
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.311

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	12.8583	12	3--1.0_mg/kg/day
A			
A	12.8417	12	1--------CONTROL
A			
A	12.8083	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: APCBLM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 0.098111
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 0.3153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	3.5667	12	2--0.1_mg/kg/day
B	3.1500	12	1--------CONTROL
B			
B	3.0500	12	3--1.0_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: FCORTEX

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 37004.91
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 193.61

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1450.00	12	2--0.1_mg/kg/day
A			
A	1430.00	12	1--------CONTROL
A			
A	1425.17	12	3--1.0_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: PCORTEX

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 8051.2
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 90.309

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1470.00	12	3--1.0_mg/kg/day
A			
A	1450.00	12	2--0.1_mg/kg/day
A			
A	1428.00	12	1--------CONTROL

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CAUDPUT

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 15449.6
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 125.1

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	2428.00	12	3--1.0_mg/kg/day
A			
A	2408.00	12	1--------CONTROL
B	2232.00	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CORPCOL

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 3655.322
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 60.85

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	366.42	12	3--1.0_mg/kg/day
B	288.00	12	1--------CONTROL
B			
B	278.42	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: HIPPO

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 7180.8
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 85.288

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	1074.00	12	3--1.0_mg/kg/day
A			
A	1066.00	12	1--------CONTROL
B	936.00	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: CEREBLL

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 65638.4
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 257.86

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	3556.0	12	1--------CONTROL
A			
A	3396.0	12	3--1.0_mg/kg/day
A			
A	3392.0	12	2--0.1_mg/kg/day

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: XGEM

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 30 MSE= 15.73333
Critical Value of Studentized Range= 3.487
Minimum Significant Difference= 3.9922

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	TRT
A	40.083	12	1--------CONTROL
A			
A	39.083	12	2--0.1_mg/kg/day
A			
A	38.500	12	3--1.0_mg/kg/day

February 1, 1999 EPA Assessment Submission

Attachment #3
Nonparametric Reanalysis of Thyroid Histopathology in Pups on PND5 from Argus (1998a) Neurodevelopmental Study

A. EPA analysis (Marcus, 1999)

ATTENTION PANEL MEMBER(S):

JOE HASEMAN
SUSAN PORTERFIELD
TOM ZOELLER

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

FROM: Allan H. Marcus, EMAG/NCEA-RTP (MD-52)

TO: Annie Jarabek, HPAG/NCEA-RTP (MD-52)

Attached is a set of statistical analyses of the histology data, provided as severity scores for both histology measures individual animals, that I received from you as a telefax from WPAFB (AFRL/HESD). A copy of these data is appended to the memo. I have corrected some errors in a draft version of 12/29/98, in response to specific comments from Dr. Joseph Haseman regarding the number of animals used in the analyses, identification of sub-groups, and expanding the methods of analysis to exact significance levels appropriate to these small sample sizes.

The raw data in the fax was converted into SYSTAT or StatXact data sets for further analyses. I can also export the data to spreadsheets or to SAS data sets, if needed. They are shown as Tables 1 to 6 in the attached memo. The changes are: (1) Table1 (level 1 at 10 mg/kg-day) frequency = 5; (2) Table 3 (level 1 severity at 0.1 mg/kg-day dose) frequency = 6, (at 10 mg/kg-day) frequency = 7; Table 6 (level 0 at 3 mg/kg-day) frequency = 1, (level 1 at 10 mg/kg-day) frequency = 2; Table 9 (levels "1 + 2" at dose 0) frequency = 6. The reported analyses were done using correct counts.

The exact small-sample Jonckheere-Terpstra test for ordered categories was used in Tables 1-5, and new Tables 1A-6A. These tests were based on assuming ordered categories of both dose and severity, hence are one-tailed tests. Monte Carlo approximation to the P-value was calculated in Table 6. Exact Fisher tests for 2X2 tables using the likelihood ratio criterion were carried out in Tables 7-12.

I understand that these analyses are based on data in the Argus rat developmental neurotoxicology study (Argus, 1998a). The 2x2 contingency table tests of association are straightforward and described in most elementary statistics texts. The logistic regression analyses in this version of SYSTAT used the iteratively reweighted least squares approach to maximum likelihood estimation described on p. 622 of the SYSTAT v. 5.0 manual (1995). These are very simple approaches, easily understood by most non-specialists. Further analyses using categorical regression methods may also be informative.

The sample sizes are on the small side for testing hypotheses. For that reason, the findings of marginal or statistically significant associations in the contingency table tests at 0.1 and 1 mg/kg-day are worrying, given that the study has small power to detect real effects of only modest magnitude. The logistic regression models are consistent with a steeper dose-response function at low doses than at high doses. The evidence as a whole leans toward a significant response at doses as low as 0.1 to 1 mg/kg-day. A larger study to look at these lower dose ranges would seem to be justified.

Attachment

Statistical Analyses of Standard Histopathological Measures of Thyroid Hypertrophy and Follicular Lumen Size Decrease in PND5 Rats

Allan H. Marcus, Statistician
National Center for Environmental Assessment – RTP

1. DATA STRUCTURE AND PURPOSE OF THE ANALYSES

The purpose of the analyses was to provide an assessment of possible trends in toxicity data provided to me by Annie Jarabek, based on the rat neurodevelopmental study data for pups postnatal on day 5 (PND5), reported in (Argus, 1998a). There were two toxicity endpoints: (1) Follicular epithelial cell hypertrophy (denoted HYPER), and (2) decrease in follicular lumen size (denoted SIZE). Both were coded on a discrete scale of increasing seriousness, as 0, 1, 2 for HYPER and 0, 1, 2, 3 for SIZE. There were separate studies for females and for males, so SEX was also a discrete variable. Each set of experiments was done at 5 dose levels: control (0 mg/kg-day), 0.1, 1, 3, and 10 mg/kg-day. DOSE effects could be evaluated either as an ordered categorical scale or as a numeric scale. Including DOSE as an ordered categorical scale allowed use of contingency table methods, whereas use of DOSE or log(DOSE) as a numeric scale allowed use of logistic regression models. These provide different but complementary information about the relationship, using elementary analytical methods.

2. TESTING ASSOCIATION IN CATEGORICAL RESPONSE DATA

The individual rat data were combined into contingency tables and entered into the SYSTAT (1995) data analysis system. The basic data tables are shown below, along with the results for tests of association with DOSE in a table with r rows and c columns as shown. The first set of tests was done by exact small-sample Jonckheere-Terpstra tests (StatXact, 1998) for association in ordered categories (DOSE, severity) for each sex and for both sexes, for both endpoints. We use the following symbols for significance: * for 0.01<P<0.05, ** for 0.001<P<0.01, *** for P<0.001, and # for 0.05 < P < 0.10. Because of the ordering assumed in both dimensions of the dose-severity relationship, all tests are one-tailed tests.

TABLE 1

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1
1	1	2	3
3	3	2	1
10	0	5	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.0811#
DF=8

TABLE 2

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1
1	2	3	1
3	1	4	1
10	0	2	4

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.0004***
DF=8

TABLE 3

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	6	2
1	3	5	4
3	4	6	2
10	0	7	5

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.0005***, DF=8

TABLE 4

SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE DOSE, mg/kg-day	LEVEL 0	1	2	3
0	2	3	1	0
0.1	1	3	2	0
1	1	4	1	0
3	1	1	2	2
10	0	2	3	1

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.0110*, DF=12

TABLE 5

SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE DOSE, mg/kg-day	LEVEL 0	1	2	3
0	4	1	1	0
0.1	1	3	2	0
1	1	1	4	0
3	0	2	4	0
10	0	0	3	3

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.0001***, DF=12

TABLE 6

SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL DOSE, mg/kg-day	LEVEL 0	1	2	3
0	6	4	2	0
0.1	2	6	4	0
1	2	5	5	0
3	1	3	6	2
10	0	2	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN ALL SEXES: 0.0000***, DF=12

We also repeated these tests for a much more focused assessment of controls vs. dose 0.1 mg/kg, using all levels of severity, but maintaining the ordering of alternatives in the exact small-sample Jonckheere-Terpstra tests. This is shown in Tables 1A-6A.

TABLE 1A

HYPERTROPHY, FEMALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	3	2	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN FEMALES: 0.4621 DF=2

TABLE 2A

HYPERTROPHY, MALES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	5	1	0
0.1	1	4	1

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION IN MALES: 0.0325*
DF=2

TABLE 3A

HYPERTROPHY, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL			
DOSE, mg/kg-day	LEVEL 0	1	2
0	9	2	1
0.1	4	6	2

P-VALUE FOR DOSE VS. HYPERTROPHY ASSOCIATION: 0.0432*, DF=2

TABLE 4A
SIZE, FEMALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, FEMALE DOSE, mg/kg-day	LEVEL 0	1	2
0	2	3	1
0.1	1	3	2

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN FEMALES: 0.3528, DF=2

TABLE 5A
SIZE, MALE: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, MALE DOSE, mg/kg-day	LEVEL 0	1	2
0	4	1	1
0.1	1	3	2

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN MALES: 0.1050, DF=2

TABLE 6A
SIZE, BOTH SEXES: NUMBER OBSERVED BY DOSE AND LEVEL

SIZE, ALL	LEVEL 0	1	2
DOSE, mg/kg-day			
0	6	4	2
0.1	2	6	4

P-VALUE FOR DOSE VS. SIZE ASSOCIATION IN BOTH SEXES: 0.0661#, DF=2

Exact Fisher tests were performed on reduced 2 by 2 tables, using DOSE level 0.1 and 1 mg/kg-day vs. controls to see if there was a significant difference at low doses. Tests of the controls against the highest 2 doses were significant and are not shown here. The low-dose tests for HYPER used a combined HYPER score of 1+2 to combine the more serious effects These tables were then combined into single tables for the purpose of providing a concise display of the results. All of the tests are one-tailed likelihood ratio tests, following a natural ordering of alternatives.

TABLE 7
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 0.1	3	3	1	5	4	8
P VALUE	0.5000		0.0400*		0.0498*	

TABLE 8
2 BY 2 CONTINENCY TABLE TESTS FOR HYPERTROPHY AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
HYPER LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	4	2	5	1	9	3
DOSE 1.0	1	5	2	4	3	9
P VALUE	0.1212		0.1212		0.0196*	

The 2 by 2 tests for SIZE effects required a more detailed level of the aggregated SIZE categories. We show separate results for category 0 vs. 1+2, and categories 0+1 vs. 2. Category 3 had no counts at dose levels 0, 0.1 and 1.

TABLE 9

2 BY 2 CONTINENCY TABLE TESTS FOR SIZE EFFECT AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 0.1	1	5	1	5	2	10
P VALUE	0.1212		0.1212		0.0965#	

TABLE 10

2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 0.1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 0.1	4	2	4	2	8	4
P VALUE	0.1212		0.1212		0.3202	

TABLE 11

2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0	1+2	0	1+2	0	1+2
DOSE 0	2	4	4	2	6	6
DOSE 1	1	5	1	5	2	10
P VALUE	0.1212		0.1212		0.0965#	

TABLE 12

2 BY 2 CONTINENCY TABLE TESTS FOR SIZE AT DOSE 1 mg/kg-day

SEX	FEMALE		MALE		ALL	
SIZE LEVEL	0+1	2	0+1	2	0+1	2
DOSE 0	5	1	5	1	10	2
DOSE 1	5	1	2	4	7	5
P VALUE	0.5000		0.1212		0.1854	

3. LOGISTIC REGRESSION ANALYSIS

As a check on the overall relationship, we also carried out logistic regression analyses of response vs. dose and vs. log(dose), for males and females separately and for both sexes combined. The dose for controls was taken as 0, and log(dose) as log(0.01 mg/kg-day). The results are shown in the following tables.

TABLE 13
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.332	0.210	-16.90
MALE	0.614	0.397	-14.78
ALL	0.423*	0.192	-32.06

TABLE 14
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.335	0.313	-12.31
MALE	1.734	1.187	-10.68
ALL	0.614	0.378	-22.30

TABLE 15
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.198#	0.109	-18.34
MALE	0.635#	0.339	-15.15
ALL	0.279***	0.097	-35.66

TABLE 16
LOGISTIC REGRESSION COEFFICIENT OF HYPERTROPHY > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.342*	0.174	-17.08
MALE	0.532**	0.207	-13.95
ALL	0.426***	0.132	-31.49

TABLE 17
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 0 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.269	0.205	-12.60
MALE	0.704**	0.284	-10.02
ALL	0.459***	0.166	-22.07

TABLE 18
LOGISTIC REGRESSION COEFFICIENT OF SIZE > 1 VS. LOG DOSE

SEX	COEFFICIENT	STD. ERROR	LOG-LIKELIHOOD
FEMALE	0.330#	0.179	-18.20
MALE	0.572**	0.208	-15.20
ALL	0.430***	0.132	-34.86

The relationship between non-transformed dose and hypertrophy is statistically significant in both sexes combined, and positive but not significant in both sexes separately. The relationship with the logarithm of dose is significant or very significant in all analyses. This suggests that the risk of a hypertrophic response increases as (roughly) the 0.3 to 0.5 power of dose. Since the dose-response function is nonlinear with a steeper slope near the origin, the possibility of significant responses at low doses is consistent with the contingency table tests.

The regression coefficients of any size > 0 vs. untransformed dose are positive but not significant, whereas after log-transformation, the effects for males and for both sexes are very significant. If the severity cutpoint for SIZE is taken as levels 2+3 vs. levels 0+1, then the relationship with dose is marginally significant in either sex and highly significant when sexes are combined. The effects for males and for both sexes combined are highly significant in the model for log of dose, which also suggests that the SIZE response probability at low doses increases as roughly the 0.3 to 0.5 power of dose.

Additional logistic regression models explored the possibility of a dose-sex interaction, with males having a steeper dose-response curve. No statistically significant gender effect was found, but it is unlikely that these small samples allow sufficient power to detect this effect.

4. SUMMARY

There appears to be strong evidence for a dose-response relationship between perchlorate dose and both endpoints, follicular epithelial cell hypertrophy and decrease in follicular lumen size. Even though the number of rats in each treatment group is smaller than is desirable to have substantial power against real effects of modest size at the two lowest dose levels, attention should be paid to the simple comparisons in Tables 2A, 3A, 7 and 8, which suggest a significant increase in hypertrophy for males, and for both groups combined at both 0.1 and 1 mg/kg-day (significant). One should note that the differences lie in the expected direction if there is a real dose-response relationship. Although there may be a dose-sex interaction, with males showing stronger effects than females, this was not significant, and combining the sexes gave evidence for an effect on follicular epithelial cell hypertrophy.

Similar analyses did not find strongly significant decreases in follicular lumen cell size at the lowest two levels using the very basic contingency table tests in Tables 9 through12, nor in Tables 4A, 5A, and 6A. However, the logistic regression models suggested that there is a very significant dose response relationship overall, with a strong model-based suggestion of a steeper dose-response relationship for lumen cell size at lower doses.

Taking the small samples sizes and limited power of these data into account, there is an indication of increased effects at levels as low as 0.1 to 1 mg/kg-day, particularly for the follicular epithelial cell hypertrophy in males.

5. REFERENCES

1. Argus, 1998a. A neurobehavioral developmental study of ammonium perchlorate administered orally in drinking water to rats [report ammendment: July 27, 1998]. Argus Research Laboratories, Inc., Horsham, PA. Argus Protocol #1613-002,

2. Wilkinson, L. SYSTAT: The System for Statistics. SYSTAT Inc., Evanston, Il., 1995.

3. StatXact Program. Cytel Inc., Cambridge, MA. 1998.

Appendix: Data as received by telefax.

February 1, 1999 EPA Assessment Submission

Attachment #4
Hormone Data Analysis for F0 and F1
from Argus (1998b) 2-Generation Reproductive Study

A. EPA analysis (Geller, 1999b)
B. EPA analysis (House, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
JOE HASEMAN
SUSAN PORTERFIELD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: February 1, 1999

Subject: Analysis of the Thyroid Hormone Data from the Rat Two Generation Reproduction Study (Argus, 1998b)

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Attached is the statistical analysis of the hormone data from the Argus Rat Developmental Neurotoxicology Study (Argus, 1998b). A memo (York, 1999g) from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) provided thyroid hormone and thyrotropin data from the oral (drinking water) two-generation reproductive study of ammonium perhclorate in the rat. Data were supplied on diskette in the form of ASCII text reports, one report for each gender/age group, and imported in ASCII form to SAS for further analysis. I have attached a description of how the analyses were done, a description of results, and summary graphs.

An alternative statistical analysis for the F1 generation, per suggestion by Joseph Haseman, is provided in the memo from Dennis House (1999) using these same data. These analyses have been provided for comparative purposes.

Analyses of Hormone Data from the Argus Oral (Drinking Water) Two-Generation Reproduction Study

Summary: A memo from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) contains thyroid hormone and thyrotrophin data from the Oral (Drinking Water) Two-Generation reproduction Study of ammonium perchlorate in the rat. The following is a statistical analysis of the thyroid and pituitary hormone data (T4, thyroxine; T3, triiodothyronine; TSH, thyroid stimulating hormone) found in that report. At the time of this analysis, data were available from both the F0 generation, females and males sacrificed at 5 and 6 months of age, respectively, and the F1 generation, one male and one female from each litter, sacrificed on postnatal day 21 (PND21). Males were sacrificed after 13 weeks of exposure, i.e., approximately 91 days. Females were sacrificed after 16 weeks, i.e. at weaning, approximately 120 days of exposure.

Data from the F0 generation were re-analyzed to look for dose and gender effects. Data from the F1 generation were re-analyzed using gender as a repeated measure within each litter. Results of these re-analyses are similar to those stated in the memo from Argus RE: Protocol 1416-001 (20 November 1998).

For the F0 generation, a NOEL of 3.0 mg/kg/day was identified from a decrease in T4 and an increase in TSH of male rats. These results are consistent with the known mechanism-of-action (MOA) of perchlorate (inhibition of thyroid hormones). The increased TSH is likely a result of the activation of the pituitary-thyroid feedback mechanism. These data are not consistent with the results of the 90-day drinking water study (Springborn Laboratories, Inc., 1998). In that study, 90 days of exposure resulted in LOELs of 0.01 mg/kg/day for T3 and T4 and a NOEL of 0.05 mg/kg/day for TSH.

For the F1 generation, a LOAEL of 0.3 mg/kg/day was identified for a decrease in TSH level, inconsistent with known MOA of perchlorate. This data is inconsistent with results from the Neurodevelopmental Toxicity Study (Argus, 1998a, Crofton, 1998f). In the Neurodevelopmental study, dose-related decreases of T4 and T3 and dose-related increase of TSH were found. Possible reasons for this disparity are discussed.

Data: All data were supplied in the form of ASCII text reports, one report for each gender/age group. Data were exported as ASCII files for analyses by SAS.

F0 generation: Data for dependent measures (T4, T3 and TSH) were subjected to separate two-way ANOVAs. Treatment (dose) and sex were the independent between-subjects variables. Mean contrasts were performed using Tukey's Studentized Range (HSD) Test. Where there was a dose x sex interaction, separate one-way ANOVAs were run for each gender.

F1 generation: Data for dependent measures (T4, T3, TSH) were subjected to separate repeated-measures ANOVAs. Treatment (dose) was the independent between-subjects variable. Sex was a within-litter repeated-measures variable. The repeated-measures analysis requires a full set of data for each litter, i.e. 1 male and 1 female. Data was missing from 4 litters (1 male from each of 0, 0.3, and 30 mg/kg/day dose groups and 1 female from 30 mg/kg/day), reducing the sample size in the analysis from 99 to 95. Mean contrasts were performed using Tukey's

Studentized Range (HSD) Test.

To correct for multiple comparisons (i.e., separate analyses for T4 and TSH) the acceptable alpha for significance (for all interaction main effects tests) was corrected to 0.029 (alpha of 0.05 divided by the square root of the number of ANOVAs). SAS analysis code and output are attached in Appendix 1.

Data Analysis - Results:

F0 Generation: There were significant dose effects for T4 and TSH, and dose x sex effects for T4 and TSH (Figure 1). Given our assumptions about the mechanism of action (MOA) of perchlorate (i.e., iodide uptake inhibition resulting in reduced levels of T4 and T3, and an increase in TSH), only the effects on T4 and TSH levels for males can be considered biologically significant. NOELs were identified for males only for T4 and TSH at a dose of 3.0 mg/kg/day. There were also significant effects of sex on T4 and T3 levels.

F1 Generation: There were no significant main effects of dose on T4, T3, or TSH. There were significant dose x sex interactions for T4 and TSH (Figure 2). The significant effect of dose on female T4 data is due to an elevated level in the 0.3 mg/kg/day group relative to the high dose group and is not consistent with the MOA of perchlorate. There was a LOEL of 0.3 mg/kg/day for a reduction in TSH level in males; this is not consistent with the known MOA of perchlorate.

These results are different from those in the F1 generation of the Neurodevelopmental Toxicity study (Argus, 1998a, Crofton, 1998f). In PND5 pups exposed through gestation and lactation, there were significant dose-related reductions in T4 and T3, and a significant dose-related increase in TSH. One possible source of this disparity is that the PND21 weanlings tested in the Two-Generation study likely received a reduced dose of the test compound through lactation (Fisher, 1998b) and the slow addition of drinking water to their diets. This may have allowed recovery from the hormone deficits due to gestational effects still visible in the younger pups.

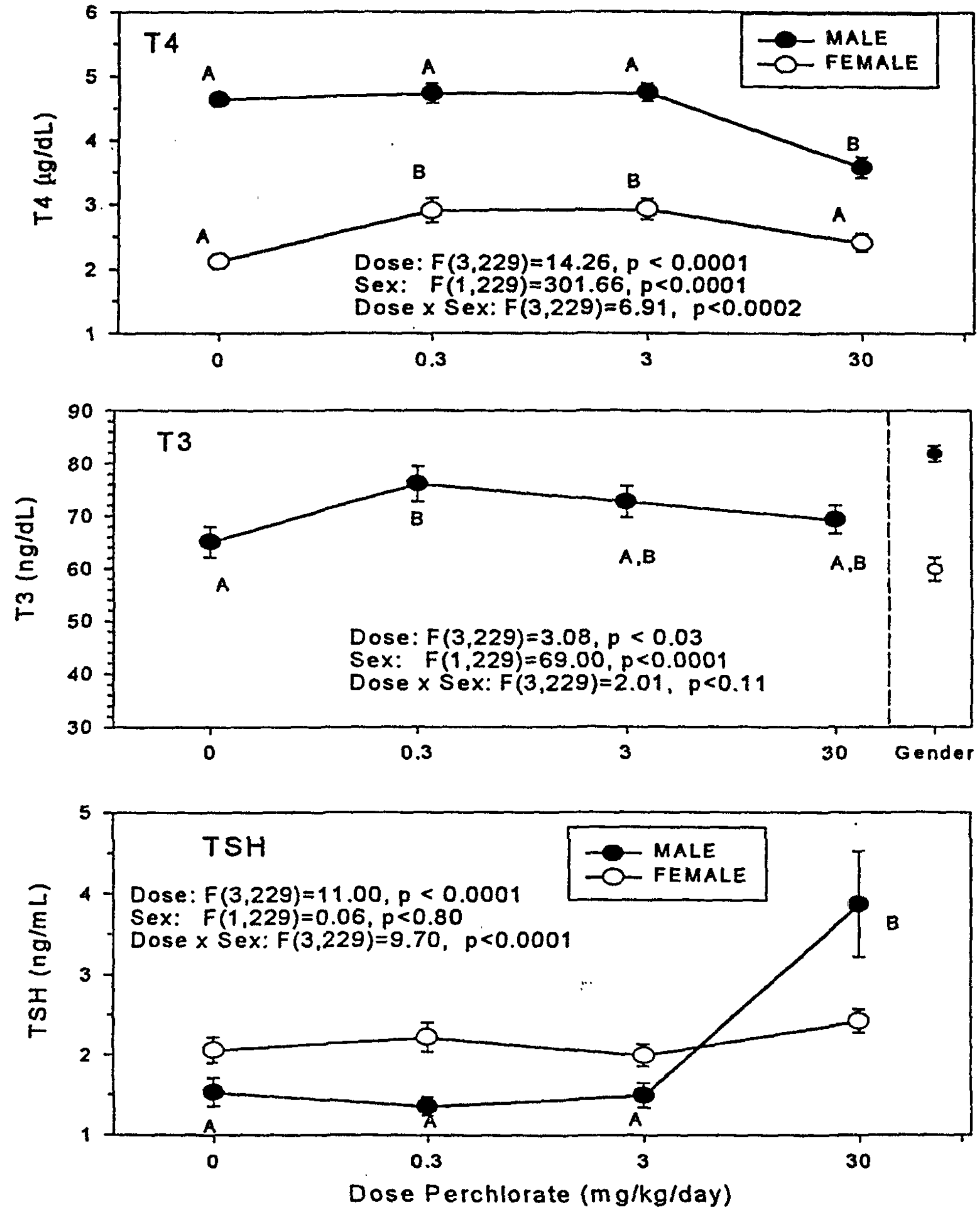
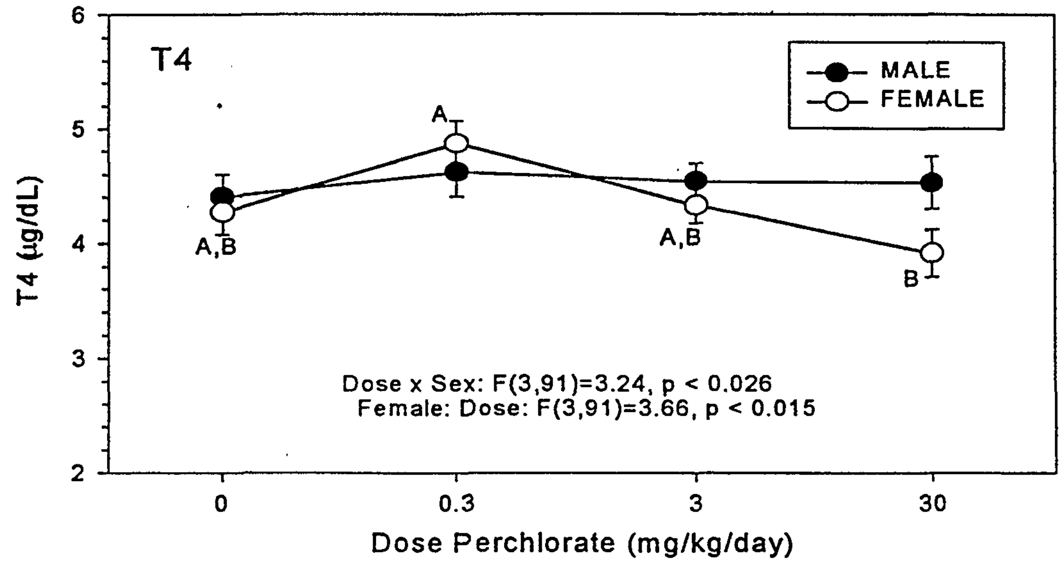


Figure
1. Effects of oral perchlorate exposure on hormone levels in F0 generation. Serum total thyroxine (T4) (top): There were significant dose, sex, and dose x sex effects. Means with different letters (on each function) were significantly different ($p<0.05$). Serum total triiodothyronine (T3)(middle): There was a significant effects of sex and a borderline significant effect of dose. Plot to right of dotted line illustrates sex effect (males>females). Serum thyroid stimulation hormone (TSH) (bottom): There were significant effects of dose and dose x sex. Means with different letters were significantly different ($p<0.05$).



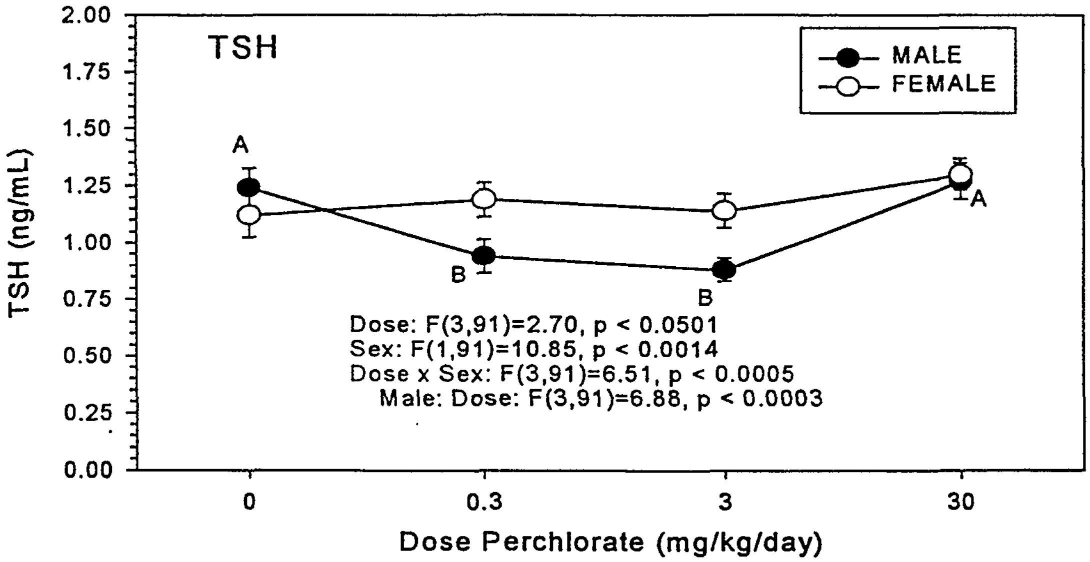


Figure 2. Effects of perchlorate exposure on hormone levels in F1 generation at post-natal day 21. Serum total thyroxine (T4) (top): There was a significant dose x sex effect, with a dose effect in females due to elevated T4 in the 0.3 mg/kg dose group. Means with different letters were significantly different ($p<0.05$). Serum thyroid stimulation hormone (TSH) (bottom): There were significant main effects of sex and dose x sex, with a dose effect in males. Means with different letters were significantly different ($p<0.05$).

APPENDIX 1 - Raw Data and Statistical Analysis

```
NOTE: Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software Release 6.12  TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.


WARNING: Your system is scheduled to expire on February 18, 1999, which is 28 days from now. Please contact your installation
         representative to have your system renewed. The SAS system will no longer function on or after that date.
 Welcome to the NHEERL-RTP SAS Information Delivery System.


           /* INPUT NEWLY RECEIVED THYROID HORMONE DATA FROM 2 GEN Reproduction STUDY.
              DATA GENERATED BY ARGUS RESEARCH LABS, RECEIVED JAN 7, 1999 */

           DATA F21; /* Female rats, generation F1,  day 21 */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
             INFILE '[GELLER.BMD]GEN2F21.DAT';
             INPUT a $ no id grp sex $ age $ tsh1 t31 t41;
             DROP a no sex;
           RUN;

NOTE: The infile '[GELLER.BMD]GEN2F21.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN2F21.DAT

NOTE: 98 records were read from the infile '[GELLER.BMD]GEN2F21.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.F21 has 98 observations and 6 variables.


           DATA M21; /* Male rats, generation F1, day 21 */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
             INFILE '[GELLER.BMD]GEN2M21.DAT';
             INPUT a $ no id grp sex $ age $ tsh2 t32 t42;
             DROP a no sex;
           RUN;

NOTE: The infile '[GELLER.BMD]GEN2M21.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN2M21.DAT

NOTE: 96 records were read from the infile '[GELLER.BMD]GEN2M21.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.M21 has 96 observations and 6 variables.


           PROC SORT DATA=f21;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
             BY id;
           RUN;

NOTE: The data set WORK.F21 has 98 observations and 6 variables.
```

```
20        PROC SORT DATA=m21;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
21          BY id;
22        RUN;

NOTE: The data set WORK.M21 has 96 observations and 6 variables.

23
24        DATA day21rep;
WARNING: The BASE Product produot with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
25          MERGE f21 m21;
26          BY id;
27        RUN;

NOTE: The data set WORK.DAY21REP has 99 observations and 9 variables.

28
29        /*****************************************************************/
30        /* For F0 generation, rats are not tracked by litter.  Therefore */
31        /* simply concatenate the male and female data sets and analyze */
32        /* with 2 way analysis of variance (grp, sex)                  */
33        /************************************************************/
34
35        DATA F5M; /* Female rats, F0, 5 months */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
36          INFILE '[GELLER.BMD]GEN25MF.DAT';
37          INPUT a $ no id grp sex $ age $ tsh t3 t4;
38          DROP a no;
39        RUN;

NOTE: The infile '[GELLER.BMD]GEN25MF.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN25MF.DAT

NOTE: 119 records were read from the infile '[GELLER.BMD]GEN25MF.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
NOTE: The data set WORK.F5M has 119 observations and 7 variables.

40
41        DATA M6M; /* Male rats, F0, 6 months */
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
42          INFILE '[GELLER.BMD]GEN26MM.DAT';
43          INPUT a $ no id grp sex $ age $ tsh t3 t4;
44          DROP a no;
45        RUN;

NOTE: The infile '[GELLER.BMD]GEN26MM.DAT' is:
      File=DSA21:[SAS$USERS.GELLER.BMD]GEN26MM.DAT

NOTE: 118 records were read from the infile '[GELLER.BMD]GEN26MM.DAT'.
      The minimum record length was 74.
      The maximum record length was 74.
```

```
NOTE: The data set WORK.M6M has 118 observations and 7 variables.

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          PROC SORT DATA=f5m;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
            BY id;
          RUN;

NOTE: The data set WORK.F5M has 119 observations and 7 variables.

          PROC SORT DATA=m6m;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
            BY id;
          RUN;

NOTE: The data set WORK.M6M has 118 observations and 7 variables.

          DATA F0;
WARNING: The BASE Product product with which DATASTEP is associated will expire within 30 days. Please contact your SAS
         installation representative to have it renewed.
            set f5m m6m;
          RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

          /******************************************************************/
          /*  Analysis of F1, by dose group with sex as a repeated measure      */
          /****************************************************************/
          PROC SORT DATA=day21rep;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
            BY grp;
          RUN;

NOTE: The data set WORK.DAY21REP has 99 observations and 9 variables.

          PROC PRINT;
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
            TITLE1'Generation F1, DAY 21 data for repeated measures.';
            TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
          RUN;

NOTE: The PROCEDURE PRINT printed pages 1-2.

          PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
            BY grp;
```

```
         VAR tshl t31 t41 tsh2 t32 t42;
         TITLE1'MEANS of Generation F1, DAY 21 data for repeated measures.';
         TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
```



```
       RUN;

NOTE: The PROCEDURE MEANS printed page 3.


       PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
        CLASS grp;
        MODEL t41 t42=grp;
        REPEATED sex 2 /SUMMARY;
        MEANS grp /TUKEY LINES;
        TITLE1'Generation F1,DAY 21, T4';
        TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
       RUN;


NOTE: The PROCEDURE GLM printed pages 4-12.

       PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
        CLASS grp;
        model t31 t32=grp;
        REPEATED sex 2 /SUMMARY;
        MEANS grp /TUKEY LINES;
        TITLE1'Generation F1,DAY 21, T3';
        TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
       RUN;


NOTE: The PROCEDURE GLM printed pages 13-21.

       PROC GLM DATA=day21rep;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
        CLASS grp;
        model tshl tsh2=grp;
        REPEATED sex 2 /SUMMARY;
        MEANS grp /TUKEY LINES;
        TITLE1'Generation F1,DAY 21, TSH';
        TITLE2'Suffix=1 for Females; Suffix=2 for Males.';
       RUN;

         /**************************************************/
        /*    ANALYSIS OF F0, BY DOSE GRP AND SEX        */
       /**************************************************/

NOTE: The PROCEDURE GLM printed pages 22-30.

       PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
```

```
         representative to have it renewed.
109         BY grp sex;
110         RUN;

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NOTE: The data set WORK.F0 has 237 observations and 7 variables.

111
112         PROC PRINT DATA= F0;
WARNING: The BASE Product product with which PRINT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
113         TITLE1'DATA FROM F0 GENERATION';
114         RUN;

NOTE: The PROCEDURE PRINT printed pages 31-35.

115
116         PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
117         BY sex;
118         RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

119
120         PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
121         BY sex;
122         TITLE'F0 Generation, Means by SEX';
123         RUN;

NOTE: The PROCEDURE MEANS printed page 36.

124
125         PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
126         BY grp;
127         RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.

128
129         PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
130         BY grp;
131         TITLE'F0 Generation, Means by Dose Group';
132         RUN;

NOTE: The PROCEDURE MEANS printed page 37.

133
134         PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
```

```
        BY grp sex;
      RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.
```

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```

      PROC MEANS N MEAN STDERR STD MIN MAX;
WARNING: The BASE Product product with which MEANS is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
        BY grp sex;
        TITLE'F0 Generation, Means by Dose and Sex';
      RUN;

NOTE: The PROCEDURE MEANS printed pages 38-39.


      PROC GLM DATA=F0;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
       CLASSES grp sex;
       model t4 t3 tsh=grp|sex;
       MEANS grp|sex /TUKEY LINES;
       TITLE'Generation F0, ADULT';
      RUN;

NOTE: Means from the MEANS statement are not adjusted for other terms in the model.  For adjusted means, use the LSMEANS statement.


NOTE: The PROCEDURE GLM printed pages 40-49.

      PROC SORT DATA=F0;
WARNING: The BASE Product product with which SORT is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
        BY sex;
      RUN;

NOTE: The data set WORK.F0 has 237 observations and 7 variables.


      PROC GLM DATA=F0;
WARNING: The SAS/STAT product with which GLM is associated will expire within 30 days. Please contact your SAS installation
         representative to have it renewed.
       BY sex;
       CLASSES grp;
       MODEL t4 t3 tsh=grp;
       MEANS grp/TUKEY LINES;
       TITLE1'Generation F0, ADULT';
       TITLE2'Analysis by Sex';
      RUN;

NOTE: Interactivity disabled with BY processing.

NOTE: The PROCEDURE GLM printed pages 50-63.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

Suffix=1 for Females; Suffix=2 for Males.

OBS	ID	GRP	AGE	TSH1	T31	T41	TSH2	T32	T42
1	3801	0.0	21D	0.54	118.93	4.57	0.90	120.00	5.66
2	3802	0.0	21D	0.56	127.92	2.80	.	.	.
3	3803	0.0	21D	1.64	93.98	4.37	0.85	99.80	4.97
4	3804	0.0	21D	0.87	112.45	3.55	1.14	104.22	3.83
5	3805	0.0	21D	1.16	114.92	5.82	0.97	112.25	4.76
6	3806	0.0	21D	0.74	95.62	4.24	1.02	98.19	4.09
7	3807	0.0	21D	1.30	107.53	4.34	0.97	104.56	4.46
8	3808	0.0	21D	1.53	100.83	4.66	1.34	109.78	5.27
9	3809	0.0	21D	1.07	107.58	4.42	1.90	86.13	3.07
10	3810	0.0	21D	0.86	102.97	4.53	1.03	99.47	5.02
11	3811	0.0	21D	0.91	122.60	4.05	1.03	110.48	4.54
12	3812	0.0	21D	1.19	104.24	3.55	1.61	99.33	4.41
13	3813	0.0	21D	1.74	103.20	3.18	1.66	116.19	3.59
14	3814	0.0	21D	0.85	109.83	3.14	1.59	118.50	5.95
15	3815	0.0	21D	0.69	88.40	2.48	0.56	103.55	2.67
16	3816	0.0	21D	2.74	85.37	3.32	2.30	96.28	3.17
17	3818	0.0	21D	0.85	104.36	4.89	1.17	116.60	4.52
18	3819	0.0	21D	1.16	101.32	3.59	0.98	94.88	3.10
19	3821	0.0	21D	0.84	79.83	4.37	0.58	111.49	3.81
20	3822	0.0	21D	0.57	90.65	3.09	0.70	97.51	3.49
21	3823	0.0	21D	1.18	107.12	3.08	1.16	105.90	2.55
22	3824	0.0	21D	1.45	117.65	4.68	1.93	108.85	5.97
23	3825	0.0	21D	0.73	104.83	5.96	1.17	115.59	4.89
24	3826	0.0	21D	1.51	105.71	5.29	1.59	85.59	3.77
25	3827	0.0	21D	1.35	115.38	5.59	1.84	114.60	5.76
26	3828	0.0	21D	0.16	137.69	6.65	1.12	125.29	5.41
27	3829	0.0	21D	1.70	115.59	4.80	1.59	106.54	5.60
28	3830	0.0	21D	1.48	90.20	4.54	0.71	97.65	4.56
29	3831	0.3	21D	0.84	118.75	5.65	1.16	140.77	5.55
30	3833	0.3	21D	1.47	122.11	6.87	1.02	102.38	3.45
31	3834	0.3	21D	0.74	105.27	4.72	0.50	98.34	3.77
32	3837	0.3	21D	1.61	109.33	4.62	1.12	109.50	4.00
33	3838	0.3	21D	0.76	116.85	3.92	0.48	104.64	3.91
34	3842	0.3	21D	0.96	116.94	4.97	0.83	116.33	4.59
35	3843	0.3	21D	0.91	123.21	5.54	1.00	126.47	4.72
36	3845	0.3	21D	0.47	95.72	4.43	0.63	77.78	3.68
37	3846	0.3	21D	1.62	99.48	4.52	0.97	100.00	4.52
38	3847	0.3	21D	1.14	139.51	4.28	.	.	.
39	3848	0.3	21D	1.11	99.10	4.77	0.97	83.62	3.07
40	3849	0.3	21D	1.33	125.37	5.77	0.89	117.49	5.07
41	3850	0.3	21D	1.84	85.45	4.11	0.72	91.03	4.41
42	3851	0.3	21D	1.16	105.31	3.56	0.98	116.70	5.70
43	3852	0.3	21D	1.27	106.74	4.42	0.58	109.25	3.97
44	3854	0.3	21D	1.27	116.06	5.47	0.91	128.06	6.23
45	3855	0.3	21D	0.89	119.82	4.15	0.66	135.82	4.82
46	3856	0.3	21D	1.00	114.33	3.58	1.04	119.85	3.95
47	3857	0.3	21D	1.52	93.59	4.84	1.48	115.96	6.37
48	3858	0.3	21D	1.73	104.69	6.25	2.00	117.18	5.60
49	3859	0.3	21D	1.30	111.68	6.70	1.05	126.16	6.00
50	3860	0.3	21D	1.20	88.98	3.89	0.77	96.81	3.53
51	3861	3.0	21D	1.19	94.02	5.40	0.38	96.85	4.73
52	3862	3.0	21D	1.28	100.02	3.30	0.88	91.83	3.51
53	3863	3.0	21D	1.67	104.22	5.34	1.07	115.12	5.69
54	3864	3.0	21D	0.70	81.32	3.63	0.95	86.32	3.81
55	3865	3.0	21D	1.15	88.17	4.35	1.20	86.70	4.04

Suffix=1 for Females; Suffix=2 for Males.

OBS	ID	GRP	AGE	TSH1	T31	T41	TSH2	T32	T42
56	3866	3	21D	1.21	104.71	4.07	0.81	114.95	4.22
57	3867	3	21D	0.61	111.35	4.11	0.67	101.21	3.34
58	3868	3	21D	1.43	109.34	3.28	0.93	85.71	5.11
59	3869	3	21D	0.98	113.15	4.25	0.99	100.07	4.33
60	3872	3	21D	1.48	117.39	5.01	1.34	117.67	4.26
61	3873	3	21D	2.15	118.55	4.18	1.27	114.37	4.86
62	3875	3	21D	1.40	102.32	4.54	0.99	135.10	5.59
63	3876	3	21D	1.62	142.56	4.44	1.38	126.16	4.60
64	3877	3	21D	1.23	125.24	3.94	0.70	127.60	4.77
65	3878	3	21D	0.70	112.91	4.06	0.63	139.04	4.65
66	3879	3	21D	0.90	109.09	3.78	0.60	114.07	3.87
67	3880	3	21D	0.67	89.02	4.96	0.56	101.60	5.67
68	3882	3	21D	0.82	116.22	6.23	0.83	123.71	5.71
69	3883	3	21D	1.01	120.88	3.90	0.80	119.86	4.95
70	3884	3	21D	1.30	131.74	3.78	1.17	120.28	4.03
71	3885	3	21D	0.62	98.85	4.46	0.72	96.82	4.53
72	3887	3	21D	0.86	110.08	4.12	0.88	126.66	4.59
73	3888	3	21D	1.00	108.36	5.20	0.69	102.72	4.37
74	3889	3	21D	1.36	108.76	5.06	0.76	90.42	5.54
75	3890	3	21D	1.18	114.05	2.70	0.72	110.42	2.56
76	3891	30	21D	1.11	101.34	3.64	1.10	103.06	4.09
77	3892	30	21D	1.47	106.42	2.84	.	.	.
78	3893	30	21D	1.01	96.20	4.49	0.74	118.93	5.40
79	3894	30	21D	1.50	110.06	3.96	1.55	107.78	4.94
80	3895	30	21D	2.05	89.95	4.46	1.68	94.67	5.01
81	3897	30	21D	1.32	94.62	3.17	1.20	101.91	4.31
82	3899	30	21D	1.29	94.64	4.82	0.95	91.54	4.67
83	3900	30	21D	1.34	95.71	2.83	1.22	86.49	2.85
84	3901	30	21D	0.60	82.98	2.91	0.74	79.34	4.10
85	3902	30	21D	1.11	95.47	3.52	1.04	94.80	3.59
86	3904	30	21D	1.11	90.80	2.94	1.01	93.58	4.68
87	3905	30	21D	.	.	.	1.71	117.53	2.64
88	3906	30	21D	1.14	87.52	3.42	0.89	100.49	4.94
89	3907	30	21D	1.54	79.28	2.67	1.63	127.13	6.03
90	3910	30	21D	2.14	124.86	4.21	2.43	127.13	6.70
91	3911	30	21D	1.19	90.74	2.88	1.02	104.62	3.62
92	3912	30	21D	1.19	102.83	3.78	1.44	104.71	2.74
93	3913	30	21D	1.70	98.04	4.67	1.62	95.74	5.66
94	3915	30	21D	1.65	106.41	4.65	1.24	118.72	5.75
95	3916	30	21D	1.10	115.86	4.31	1.18	101.66	3.69
96	3917	30	21D	0.93	97.81	5.24	1.28	115.02	5.51
97	3918	30	21D	1.01	93.00	4.54	1.36	142.11	5.34
98	3919	30	21D	1.34	80.96	6.74	0.98	104.32	4.04
99	3920	30	21D	1.09	108.86	3.31	1.20	138.88	3.78

GRP=0

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	28	1.1203571	0.0964152	0.5101814	0.1600000	2.7400000
T31	28	105.9535714	2.4708986	13.0747665	79.8300000	137.6900000
T41	28	4.2696429	0.1926517	1.0194170	2.4800000	6.6500000
TSH2	27	1.2374074	0.0861880	0.4478461	0.5600000	2.3000000
T32	27	105.8970370	1.9197980	9.9755631	85.5900000	125.2900000
T42	27	4.4033333	0.1950272	1.0133911	2.5500000	5.9700000

GRP=0.3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	22	1.1881818	0.0750574	0.3520503	0.4700000	1.8400000
T31	22	109.9222727	2.7857661	13.0664011	85.4500000	139.5100000
T41	22	4.8650000	0.2016799	0.9459626	3.5600000	6.8700000
TSH2	21	0.9409524	0.0745396	0.3415831	0.4800000	2.0000000
T32	21	111.1495238	3.5726718	16.3720391	77.7800000	140.7700000
T42	21	4.6147619	0.2137150	0.9793652	3.0700000	6.3700000

GRP=3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	25	1.1408000	0.0749376	0.3746879	0.6100000	2.1500000
T31	25	109.2928000	2.7136494	13.5682468	81.3200000	142.5600000
T41	25	4.3236000	0.1555141	0.7775704	2.7000000	6.2300000
TSH2	25	0.8768000	0.0508747	0.2543737	0.3800000	1.3800000
T32	25	109.8104000	3.1385677	15.6928385	85.7100000	139.0400000
T42	25	4.5332000	0.1577939	0.7889694	2.5600000	5.7100000

GRP=30

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
TSH1	23	1.3013043	0.0732916	0.3514943	0.6000000	2.1400000
T31	23	97.5808696	2.3031636	11.0455844	79.2800000	124.8600000
T41	23	3.9130435	0.2049751	0.9830261	2.6700000	6.7400000
TSH2	23	1.2700000	0.0795342	0.3814327	0.7400000	2.4300000
T32	23	107.3982609	3.3487192	16.0598932	79.3400000	142.1100000
T42	23	4.5252174	0.2264661	1.0860934	2.6400000	6.7000000

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: T41

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.51553209	3.17184403	3.66	0.0153
Error	91	78.81858370	0.86613828		
Corrected Total	94	88.33411579			

R-Square	C.V.	Root MSE	T41 Mean
0.107722	21.31723	0.93066551	4.36578947

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	9.51553209	3.17184403	3.66	0.0153

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	9.51553209	3.17184403	3.66	0.0153

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Dependent Variable: T42

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.72784090	0.24261363	0.27	0.8499
Error	91	83.05904963	0.91273681		
Corrected Total	94	83.78689053			

R-Square	C.V.	Root MSE	T42 Mean
0.008687	21.07913	0.95537260	4.53231579

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.72784090	0.24261363	0.27	0.8499

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.72784090	0.24261363	0.27	0.8499

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	T41	T42
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.97207577	2.6141	1	91	0.1094
Pillai's Trace	0.02792423	2.6141	1	91	0.1094
Hotelling-Lawley Trace	0.02872639	2.6141	1	91	0.1094
Roy's Greatest Root	0.02872639	2.6141	1	91	0.1094

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.90340906	3.2432	3	91	0.0256
Pillai's Trace	0.09659094	3.2432	3	91	0.0256
Hotelling-Lawley Trace	0.10691828	3.2432	3	91	0.0256
Roy's Greatest Root	0.10691828	3.2432	3	91	0.0256

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	5.48008956	1.82669652	1.42	0.2430
Error	91	117.32693992	1.28930703		

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted Pr > F G - G	H - F
SEX	1	1.27978048	1.27978048	2.61	0.1094	.	.
SEX*GRP	3	4.76328344	1.58776115	3.24	0.0256	.	.
Error(SEX)	91	44.55069341	0.48956806				

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	2.55956097	2.55956097	2.61	0.1094
GRP	3	9.52656687	3.17552229	3.24	0.0256
Error	91	89.10138681	0.97913612		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T41

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.866138
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.7104
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	4.8929	21	0.3
	A			
B	A	4.3241	27	0
B	A			
B	A	4.3236	25	3
B				
B		3.9618	22	30

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T42

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.912737
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.7292
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	4.6148	21	0.3
A			
A	4.6109	22	30
A			
A	4.5332	25	3
A			
A	4.4033	27	0

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: T31

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2064.38182225	688.12727408	4.54	0.0052
Error	91	13804.23115248	151.69484783		
Corrected Total	94	15868.61297474			

	R-Square	C.V.	Root MSE	T31 Mean
	0.130092	11.71489	12.31644623	105.13494737

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	2064.38182225	688.12727408	4.54	0.0052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	2064.38182225	688.12727408	4.54	0.0052

General Linear Models Procedure

Dependent Variable: T32

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	424.20015417	141.40005139	0.66	0.5773
Error	91	19425.47154057	213.46672023		
Corrected Total	94	19849.67169474			

	R-Square	C.V.	Root MSE	T32 Mean
	0.021371	13.48716	14.61050034	108.32894737

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	424.20015417	141.40005139	0.66	0.5773

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	424.20015417	141.40005139	0.66	0.5773

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	T31	T32
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.93817904	5.9964	1	91	0.0163
Pillai's Trace	0.06182096	5.9964	1	91	0.0163
Hotelling-Lawley Trace	0.06589462	5.9964	1	91	0.0163
Roy's Greatest Root	0.06589462	5.9964	1	91	0.0163

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.92796295	2.3548	3	91	0.0772
Pillai's Trace	0.07203705	2.3548	3	91	0.0772
Hotelling-Lawley Trace	0.07762923	2.3548	3	91	0.0772
Roy's Greatest Root	0.07762923	2.3548	3	91	0.0772

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1841.56332307	613.85444102	2.24	0.0885
Error	91	24894.97280640	273.57112974		

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted Pr > F G - G	H - F
SEX	1	549.21389263	549.21389263	6.00	0.0163	.	.
SEX*GRP	3	647.01865335	215.67288445	2.35	0.0772	.	.
Error(SEX)	91	8334.72988665	91.59043831				

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	1098.42778526	1098.42778526	6.00	0.0163
GRP	3	1294.03730671	431.34576890	2.35	0.0772
Error	91	16669.45977329	183.18087663		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T31

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 151.6948
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 9.4008
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	109.293	25	3
	A			
	A	108.513	21	0.3
	A			
B	A	105.140	27	0
B				
B		97.179	22	30

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T32

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 213.4667
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 11.152
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	111.150	21	0.3
A			
A	109.810	25	3
A			
A	106.938	22	30
A			
A	105.897	27	0

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in data set = 99

NOTE: Observations with missing values will not be included in this analysis. Thus, only 95 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: TSH1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.36063343	0.12021114	0.71	0.5472
Error	91	15.36005500	0.16879181		
Corrected Total	94	15.72068842			

R-Square	C.V.	Root MSE	TSH1 Mean
0.022940	34.60419	0.41084281	1.18726316

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.36063343	0.12021114	0.71	0.5472

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.36063343	0.12021114	0.71	0.5472

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure

Dependent Variable: TSH2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2.74305548	0.91435183	6.88	0.0003
Error	91	12.09964347	0.13296312		
Corrected Total	94	14.84269895			

R-Square	C.V.	Root MSE	TSH2 Mean
0.184808	33.76635	0.36464108	1.07989474

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	2.74305548	0.91435183	6.88	0.0003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	2.74305548	0.91435183	6.88	0.0003

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Repeated Measures Level Information

Dependent Variable	TSH1	TSH2
Level of SEX	1	2

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX Effect
H = Type III SS&CP Matrix for SEX E = Error SS&CP Matrix

S=1 M=-0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.89351379	10.8451	1	91	0.0014
Pillai's Trace	0.10648621	10.8451	1	91	0.0014
Hotelling-Lawley Trace	0.11917691	10.8451	1	91	0.0014
Roy's Greatest Root	0.11917691	10.8451	1	91	0.0014

Manova Test Criteria and Exact F Statistics for the Hypothesis of no SEX*GRP Effect
H = Type III SS&CP Matrix for SEX*GRP E = Error SS&CP Matrix

S=1 M=0.5 N=44.5

Statistic	Value	F	Num DF	Den DF	Pr > F
Wilks' Lambda	0.82339898	6.5058	3	91	0.0005
Pillai's Trace	0.17660102	6.5058	3	91	0.0005
Hotelling-Lawley Trace	0.21447806	6.5058	3	91	0.0005
Roy's Greatest Root	0.21447806	6.5058	3	91	0.0005

General Linear Models Procedure
Repeated Measures Analysis of Variance
Tests of Hypotheses for Between Subjects Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1.98018483	0.66006161	2.70	0.0501
Error	91	22.22138149	0.24419101		

Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Univariate Tests of Hypotheses for Within Subject Effects

Source	DF	Type III SS	Mean Square	F Value	Pr > F	Adjusted Pr > F G - G	H - F
SEX	1	0.62428642	0.62428642	10.85	0.0014	.	.
SEX*GRP	3	1.12350407	0.37450136	6.51	0.0005	.	.
Error(SEX)	91	5.23831698	0.05756392				

1 Generation F1,DAY 21, TSH 09:23 Thursday, January 21, 1999 28
Suffix=1 for Females; Suffix=2 for Males.

General Linear Models Procedure
Repeated Measures Analysis of Variance
Analysis of Variance of Contrast Variables

SEX.N represents the contrast between the nth level of SEX and the last

Contrast Variable: SEX.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MEAN	1	1.24857284	1.24857284	10.85	0.0014
GRP	3	2.24700815	0.74900272	6.51	0.0005
Error	91	10.47663396	0.11512785		

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH1

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.168792
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.3136
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	1.2936	22	30
A			
A	1.1905	21	0.3
A			
A	1.1411	27	0
A			
A	1.1408	25	3

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH2

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 91 MSE= 0.132963
Critical Value of Studentized Range= 3.701
Minimum Significant Difference= 0.2783
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 23.51411

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	1.2500	22	30
A			
A	1.2374	27	0
B	0.9410	21	0.3
B			
B	0.8768	25	3

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
1	3801	0	F	5M	0.72	27.62	1.79
2	3802	0	F	5M	3.51	88.20	4.35
3	3803	0	F	5M	0.84	23.88	1.05
4	3804	0	F	5M	1.66	55.70	1.45
5	3805	0	F	5M	1.31	56.03	2.47
6	3806	0	F	5M	2.46	36.57	2.23
7	3807	0	F	5M	1.82	48.65	2.04
8	3808	0	F	5M	1.59	62.75	2.98
9	3809	0	F	5M	1.39	62.30	2.02
10	3810	0	F	5M	2.41	44.70	2.80
11	3811	0	F	5M	1.44	50.48	2.06
12	3812	0	F	5M	1.86	47.12	1.68
13	3813	0	F	5M	2.77	43.52	2.43
14	3814	0	F	5M	1.36	52.48	2.16
15	3815	0	F	5M	1.80	68.54	2.10
16	3816	0	F	5M	3.16	108.80	1.58
17	3817	0	F	5M	1.31	68.48	2.64
18	3818	0	F	5M	1.50	159.25	1.45
19	3819	0	F	5M	2.59	41.89	1.45
20	3820	0	F	5M	1.59	114.68	3.17
21	3821	0	F	5M	2.08	44.07	1.11
22	3822	0	F	5M	5.24	60.97	1.93
23	3823	0	F	5M	1.97	43.95	1.90
24	3824	0	F	5M	1.83	24.39	1.34
25	3825	0	F	5M	2.05	53.42	1.88
26	3826	0	F	5M	2.41	40.63	2.33
27	3827	0	F	5M	2.00	38.53	1.83
28	3828	0	F	5M	2.20	47.03	2.46
29	3829	0	F	5M	2.82	60.18	2.44
30	3830	0	F	5M	1.93	58.28	2.67
31	3601	0	M	6M	1.19	88.35	5.07
32	3602	0	M	6M	0.93	84.17	4.35
33	3603	0	M	6M	0.62	79.49	5.66
34	3604	0	M	6M	0.80	82.88	5.33
35	3605	0	M	6M	2.16	86.13	5.19
36	3606	0	M	6M	1.18	93.42	4.36
37	3607	0	M	6M	1.05	63.72	3.81
38	3608	0	M	6M	1.80	69.21	4.18
39	3609	0	M	6M	4.41	72.29	4.12
40	3610	0	M	6M	1.40	68.40	4.82
41	3611	0	M	6M	0.84	61.11	4.24
42	3612	0	M	6M	1.45	61.69	4.84
43	3613	0	M	6M	0.56	71.49	4.94
44	3614	0	M	6M	1.81	83.01	4.56
45	3615	0	M	6M	1.08	77.97	5.39
46	3616	0	M	6M	3.04	69.67	3.84
47	3617	0	M	6M	1.34	71.30	4.95
48	3618	0	M	6M	2.08	51.76	3.18
49	3619	0	M	6M	0.42	68.45	4.49
50	3620	0	M	6M	0.95	66.34	5.01
51	3621	0	M	6M	2.42	62.40	4.30
52	3622	0	M	6M	1.48	96.45	4.44
53	3623	0	M	6M	3.67	61.89	4.17
54	3624	0	M	6M	0.35	73.45	4.35
55	3625	0	M	6M	1.88	80.55	4.11
56	3627	0	M	6M	1.35	55.93	5.37

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
57	3628	0.0	M	6M	0.90	65.47	5.25
58	3629	0.0	M	6M	0.75	59.35	4.87
59	3630	0.0	M	6M	2.46	77.51	5.40
60	3831	0.3	F	5M	1.46	46.98	1.38
61	3832	0.3	F	5M	2.99	76.19	3.82
62	3833	0.3	F	5M	1.42	118.14	2.13
63	3834	0.3	F	5M	1.40	39.12	3.15
64	3835	0.3	F	5M	1.64	78.71	4.42
65	3836	0.3	F	5M	1.48	102.72	3.65
66	3837	0.3	F	5M	1.30	50.23	3.71
67	3838	0.3	F	5M	2.02	54.58	2.16
68	3839	0.3	F	5M	1.16	82.60	4.07
69	3840	0.3	F	5M	2.40	132.21	5.87
70	3841	0.3	F	5M	1.71	67.83	3.55
71	3842	0.3	F	5M	2.67	50.68	2.93
72	3843	0.3	F	5M	4.82	42.96	2.44
73	3844	0.3	F	5M	1.88	141.56	3.76
74	3845	0.3	F	5M	1.07	43.53	1.14
75	3846	0.3	F	5M	1.26	49.05	2.57
76	3847	0.3	F	5M	1.63	84.55	2.96
77	3848	0.3	F	5M	2.78	54.17	2.11
78	3849	0.3	F	5M	1.13	43.88	2.48
79	3850	0.3	F	5M	2.72	46.08	2.92
80	3851	0.3	F	5M	3.71	45.33	1.66
81	3852	0.3	F	5M	1.76	34.24	1.54
82	3853	0.3	F	5M	2.51	101.45	3.29
83	3854	0.3	F	5M	1.34	42.79	2.46
84	3855	0.3	F	5M	1.62	28.25	1.42
85	3856	0.3	F	5M	3.47	40.06	3.48
86	3857	0.3	F	5M	2.79	46.68	3.64
87	3858	0.3	F	5M	4.32	69.99	2.02
88	3859	0.3	F	5M	3.17	58.12	2.98
89	3860	0.3	F	5M	2.77	70.99	3.39
90	3631	0.3	M	6M	1.83	79.54	4.79
91	3632	0.3	M	6M	0.91	77.36	4.51
92	3633	0.3	M	6M	1.30	131.87	7.01
93	3634	0.3	M	6M	0.81	79.14	4.28
94	3635	0.3	M	6M	2.09	110.78	6.17
95	3636	0.3	M	6M	1.02	79.79	4.55
96	3637	0.3	M	6M	1.69	98.71	4.28
97	3638	0.3	M	6M	0.82	107.52	4.74
98	3639	0.3	M	6M	2.38	82.07	4.28
99	3640	0.3	M	6M	2.14	80.26	3.94
100	3641	0.3	M	6M	1.82	57.22	3.30
101	3642	0.3	M	6M	1.79	87.48	3.75
102	3643	0.3	M	6M	0.65	120.54	4.83
103	3644	0.3	M	6M	1.67	83.31	4.74
104	3645	0.3	M	6M	2.64	91.05	5.01
105	3646	0.3	M	6M	1.08	102.18	5.81
106	3647	0.3	M	6M	1.42	79.21	4.57
107	3648	0.3	M	6M	1.80	72.73	4.18
108	3649	0.3	M	6M	1.26	68.76	4.86
109	3650	0.3	M	6M	0.71	89.35	5.05
110	3651	0.3	M	6M	0.72	78.85	4.25
111	3652	0.3	M	6M	0.61	92.34	5.56
112	3653	0.3	M	6M	2.29	91.63	5.07

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
113	3654	0.3	M	6M	0.31	109.01	5.63
114	3655	0.3	M	6M	0.78	80.77	4.05
115	3656	0.3	M	6M	1.08	70.67	3.80
116	3657	0.3	M	6M	1.43	91.65	5.36
117	3658	0.3	M	6M	1.17	73.19	4.18
118	3659	0.3	M	6M	0.29	74.18	3.57
119	3660	0.3	M	6M	2.07	80.52	5.66
120	3861	3.0	F	5M	1.71	47.40	3.05
121	3862	3.0	F	5M	3.25	65.02	3.67
122	3863	3.0	F	5M	0.87	61.89	2.24
123	3864	3.0	F	5M	0.62	47.74	1.36
124	3865	3.0	F	5M	2.36	42.31	2.07
125	3866	3.0	F	5M	1.46	49.32	2.56
126	3867	3.0	F	5M	2.72	49.26	2.15
127	3868	3.0	F	5M	2.26	48.82	2.20
128	3869	3.0	F	5M	1.09	45.46	2.24
129	3870	3.0	F	5M	1.80	89.35	3.60
130	3871	3.0	F	5M	1.52	85.38	4.03
131	3872	3.0	F	5M	2.11	58.63	2.69
132	3873	3.0	F	5M	2.29	45.69	2.68
133	3874	3.0	F	5M	1.92	90.35	3.97
134	3875	3.0	F	5M	2.16	53.03	3.06
135	3876	3.0	F	5M	2.88	42.69	1.46
136	3877	3.0	F	5M	3.26	52.51	2.74
137	3878	3.0	F	5M	1.50	63.21	4.25
138	3879	3.0	F	5M	1.27	38.87	1.64
139	3880	3.0	F	5M	1.44	53.24	2.68
140	3882	3.0	F	5M	1.41	58.15	2.82
141	3883	3.0	F	5M	3.87	46.05	2.90
142	3884	3.0	F	5M	1.19	75.50	3.59
143	3885	3.0	F	5M	1.44	43.87	3.15
144	3886	3.0	F	5M	2.53	70.42	4.63
145	3887	3.0	F	5M	2.46	47.75	3.83
146	3888	3.0	F	5M	1.38	50.40	2.42
147	3889	3.0	F	5M	2.27	59.71	3.69
148	3890	3.0	F	5M	2.67	52.14	3.43
149	3661	3.0	M	6M	1.29	81.05	5.05
150	3662	3.0	M	6M	2.84	99.22	3.73
151	3663	3.0	M	6M	1.54	92.30	6.09
152	3664	3.0	M	6M	1.36	102.47	4.29
153	3665	3.0	M	6M	1.71	102.42	5.34
154	3666	3.0	M	6M	0.68	67.87	4.79
155	3667	3.0	M	6M	1.39	88.38	4.39
156	3668	3.0	M	6M	0.39	68.59	4.96
157	3669	3.0	M	6M	1.22	75.98	5.99
158	3670	3.0	M	6M	1.91	98.88	4.76
159	3671	3.0	M	6M	3.76	82.85	4.58
160	3672	3.0	M	6M	0.87	90.33	5.27
161	3673	3.0	M	6M	1.40	90.58	4.17
162	3674	3.0	M	6M	1.32	90.37	4.38
163	3675	3.0	M	6M	1.03	84.18	4.57
164	3676	3.0	M	6M	0.90	72.26	4.74
165	3677	3.0	M	6M	0.87	79.72	4.73
166	3678	3.0	M	6M	0.38	69.36	3.34
167	3679	3.0	M	6M	2.47	85.77	3.79
168	3680	3.0	M	6M	1.36	74.30	5.45

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
169	3681	3	M	6M	2.58	92.89	4.40
170	3682	3	M	6M	2.19	98.91	6.02
171	3683	3	M	6M	2.07	111.73	4.99
172	3684	3	M	6M	0.77	70.66	4.27
173	3685	3	M	6M	0.45	166.80	3.47
174	3686	3	M	6M	3.03	81.70	5.52
175	3687	3	M	6M	0.96	81.88	4.39
176	3688	3	M	6M	1.47	94.46	6.49
177	3689	3	M	6M	1.05	79.94	4.74
178	3690	3	M	6M	1.35	77.72	3.62
179	3891	30	F	5M	2.33	37.30	1.90
180	3892	30	F	5M	2.04	56.00	3.92
181	3893	30	F	5M	1.27	45.02	3.02
182	3894	30	F	5M	2.99	43.84	1.83
183	3895	30	F	5M	2.59	40.47	3.06
184	3896	30	F	5M	1.34	68.29	3.24
185	3897	30	F	5M	1.20	55.97	2.39
186	3898	30	F	5M	2.67	108.40	3.11
187	3899	30	F	5M	1.52	60.10	3.29
188	3900	30	F	5M	1.51	42.03	2.28
189	3901	30	F	5M	1.44	38.32	1.83
190	3902	30	F	5M	1.27	46.37	2.25
191	3903	30	F	5M	3.33	89.67	2.39
192	3904	30	F	5M	1.66	54.31	2.59
193	3905	30	F	5M	2.47	91.70	4.62
194	3906	30	F	5M	3.02	47.31	2.46
195	3907	30	F	5M	2.64	64.16	1.88
196	3908	30	F	5M	2.14	107.67	2.78
197	3909	30	F	5M	1.80	100.45	2.54
198	3910	30	F	5M	3.30	49.36	1.58
199	3911	30	F	5M	1.53	79.20	2.98
200	3912	30	F	5M	1.80	88.74	1.59
201	3913	30	F	5M	3.06	41.83	1.57
202	3914	30	F	5M	1.51	77.67	2.47
203	3915	30	F	5M	3.58	50.85	1.08
204	3916	30	F	5M	1.45	47.37	1.53
205	3917	30	F	5M	1.82	47.81	2.06
206	3918	30	F	5M	2.49	42.50	2.93
207	3919	30	F	5M	1.98	39.32	2.28
208	3920	30	F	5M	3.46	49.17	1.19
209	3691	30	M	6M	2.58	64.35	3.66
210	3692	30	M	6M	3.24	89.66	3.66
211	3693	30	M	6M	2.70	91.40	3.10
212	3694	30	M	6M	5.17	95.93	3.94
213	3695	30	M	6M	3.36	85.61	3.95
214	3696	30	M	6M	1.28	83.01	4.05
215	3697	30	M	6M	8.21	100.77	6.08
216	3699	30	M	6M	2.07	60.94	3.23
217	3700	30	M	6M	1.94	59.68	2.63
218	3701	30	M	6M	1.76	81.16	3.52
219	3702	30	M	6M	3.82	92.08	3.39
220	3703	30	M	6M	3.74	98.18	3.63
221	3704	30	M	6M	1.76	80.75	4.37
222	3705	30	M	6M	2.29	68.34	2.62
223	3706	30	M	6M	1.97	74.23	3.00
224	3707	30	M	6M	5.37	61.49	3.27

OBS	ID	GRP	SEX	AGE	TSH	T3	T4
225	3708	30	M	6M	1.13	84.65	2.10
226	3709	30	M	6M	14.15	71.72	1.57
227	3710	30	M	6M	13.31	74.04	2.99
228	3711	30	M	6M	1.14	71.23	4.50
229	3712	30	M	6M	1.93	70.55	4.18
230	3713	30	M	6M	7.40	71.35	4.45
231	3714	30	M	6M	1.05	64.68	3.00
232	3715	30	M	6M	1.79	116.75	4.08
233	3716	30	M	6M	1.97	89.85	4.68
234	3717	30	M	6M	0.94	66.63	3.19
235	3718	30	M	6M	8.27	78.42	3.95
236	3719	30	M	6M	6.87	54.88	3.35
237	3720	30	M	6M	1.04	76.21	3.61

SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	119	3860.33	3.1975080	34.8806948	3801.00	3920.00
GRP	119	8.3697479	1.1609977	12.6649895	0	30.0000000
TSH	119	2.1087395	0.0773778	0.8440927	0.6200000	5.2400000
T3	119	59.8497479	2.2107705	24.1166592	23.8800000	159.2500000
T4	119	2.5910084	0.0827259	0.9024331	1.0500000	5.8700000

SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	118	3660.47	3.2002262	34.7633543	3601.00	3720.00
GRP	118	8.2118644	1.1549327	12.5457806	0	30.0000000
TSH	118	2.0492373	0.1953372	2.1219049	0.2900000	14.1500000
T3	118	81.8444068	1.5246098	16.5615013	51.7600000	166.8000000
T4	118	4.4274576	0.0832387	0.9042040	1.5700000	7.0100000

GRP=0

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3717.02	13.2011036	101.3996007	3601.00	3830.00
TSH	59	1.7964407	0.1230441	0.9451195	0.3500000	5.2400000
T3	59	65.0328814	2.9519008	22.6739804	23.8800000	159.2500000
T4	59	3.3623729	0.1841824	1.4147320	1.0500000	5.6600000

GRP=0.3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	60	3745.50	13.0675667	101.2209364	3631.00	3860.00
TSH	60	1.7830000	0.1202247	0.9312563	0.2900000	4.8200000
T3	60	76.0891667	3.3691531	26.0973476	28.2500000	141.5600000
T4	60	3.8146667	0.1684937	1.3051469	1.1400000	7.0100000

GRP=3

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3773.71	13.1659384	101.1294921	3661.00	3890.00
TSH	59	1.7342373	0.1077443	0.8275998	0.3800000	3.8700000
T3	59	72.6733898	2.9975333	23.0244899	38.8700000	166.8000000
T4	59	3.8494915	0.1592210	1.2230000	1.3600000	6.4900000

GRP=30

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	59	3807.32	13.1611298	101.0925559	3691.00	3920.00
TSH	59	3.0077966	0.3421046	2.6277551	0.9400000	14.1500000
T3	59	69.3176271	2.6843263	20.6187013	37.3000000	116.7500000
T4	59	2.9896610	0.1308737	1.0052602	1.0800000	6.0800000

GRP=0 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3815.50	1.6072751	8.8034084	3801.00	3830.00
TSH	30	2.0540000	0.1594023	0.8730825	0.7200000	5.2400000
T3	30	57.7696667	5.1493264	28.2040220	23.8800000	159.2500000
T4	30	2.1263333	0.1236620	0.6773248	1.0500000	4.3500000

GRP=0 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3615.14	1.6209255	8.7289508	3601.00	3630.00
TSH	29	1.5300000	0.1777452	0.9571871	0.3500000	4.4100000
T3	29	72.5465517	2.0850083	11.2281134	51.7600000	96.4500000
T4	29	4.6410345	0.1083501	0.5834830	3.1800000	5.6600000

GRP=0.3 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3845.50	1.6072751	8.8034084	3831.00	3860.00
TSH	30	2.2133333	0.1801745	0.9868561	1.0700000	4.8200000
T3	30	64.7890000	5.3452191	29.2769705	28.2500000	141.5600000
T4	30	2.9033333	0.1896520	1.0387670	1.1400000	5.8700000

GRP=0.3 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3645.50	1.6072751	8.8034084	3631.00	3660.00
TSH	30	1.3526667	0.1165263	0.6382408	0.2900000	2.6400000
T3	30	87.3893333	2.9681270	16.2571009	57.2200000	131.8700000
T4	30	4.7260000	0.1492407	0.8174249	3.3000000	7.0100000

GRP=3 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3875.31	1.6520650	8.8966424	3861.00	3890.00
TSH	29	1.9900000	0.1435424	0.7729997	0.6200000	3.8700000
T3	29	56.3503448	2.5982097	13.9917875	38.8700000	90.3500000
T4	29	2.9241379	0.1562495	0.8414296	1.3600000	4.6300000

GRP=3 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3675.50	1.6072751	8.8034084	3661.00	3690.00
TSH	30	1.4870000	0.1488030	0.8150276	0.3800000	3.7600000
T3	30	88.4523333	3.4021201	18.6341793	67.8700000	166.8000000
T4	30	4.7440000	0.1442050	0.7898433	3.3400000	6.4900000

GRP=30 SEX=F

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	30	3905.50	1.6072751	8.8034084	3891.00	3920.00
TSH	30	2.1736667	0.1357876	0.7437393	1.2000000	3.5800000
T3	30	60.3733333	4.0112100	21.9703018	37.3000000	108.4000000
T4	30	2.4213333	0.1445999	0.7920063	1.0800000	4.6200000

GRP=30 SEX=M

Variable	N	Mean	Std Error	Std Dev	Minimum	Maximum
ID	29	3705.76	1.6420094	8.8424915	3691.00	3720.00
TSH	29	3.8706897	0.6489744	3.4948339	0.9400000	14.1500000
T3	29	78.5703448	2.6672331	14.3634900	54.8800000	116.7500000
T4	29	3.5775862	0.1596908	0.8599612	1.5700000	6.0800000

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3
SEX	2	F M

Number of observations in data set = 237

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	241.25464107	34.46494872	52.50	0.0001
Error	229	150.32005345	0.65641945		
Corrected Total	236	391.57469451			

R-Square	C.V.	Root MSE	T4 Mean
0.616114	23.11310	0.81019717	3.50535865

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	29.62445542	9.87481847	15.04	0.0001
SEX	1	198.02136032	198.02136032	301.67	0.0001
GRP*SEX	3	13.60882533	4.53627511	6.91	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	28.07875642	9.35958547	14.26	0.0001
SEX	1	198.01581342	198.01581342	301.66	0.0001
GRP*SEX	3	13.60882533	4.53627511	6.91	0.0002

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	34937.85388704	4991.12198386	12.10	0.0001
Error	229	94446.22560494	412.42893277		
Corrected Total	236	129384.07949198			

R-Square	C.V.	Root MSE	T3 Mean
0.270032	28.68383	20.30834638	70.80067511

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	3977.55083365	1325.85027788	3.21	0.0237
SEX	1	28467.84975054	28467.84975054	69.02	0.0001
GRP*SEX	3	2492.45330286	830.81776762	2.01	0.1127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	3811.29675945	1270.43225315	3.08	0.0283
SEX	1	28458.69687677	28458.69687677	69.00	0.0001
GRP*SEX	3	2492.45330286	830.81776762	2.01	0.1127

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	129.23442772	18.46206110	8.77	0.0001
Error	229	481.83968621	2.10410343		
Corrected Total	236	611.07411392			

R-Square	C.V.	Root MSE	TSH Mean
0.211487	69.76784	1.45055280	2.07911392

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	67.87744714	22.62581571	10.75	0.0001
SEX	1	0.15498515	0.15498515	0.07	0.7863
GRP*SEX	3	61.20199542	20.40066514	9.70	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	69.42116553	23.14038851	11.00	0.0001
SEX	1	0.13455387	0.13455387	0.06	0.8006
GRP*SEX	3	61.20199542	20.40066514	9.70	0.0001

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 0.656419
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 0.3852
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.8495	59	3
A			
A	3.8147	60	0.3
B	3.3624	59	0
B			
B	2.9897	59	30

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 412.4289
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 9.6566
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	76.089	60	0.3
	A			
B	A	72.673	59	3
B	A			
B	A	69.318	59	30
B				
B		65.033	59	0

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 2.104103
Critical Value of Studentized Range= 3.660
Minimum Significant Difference= 0.6897
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 59.24686

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.0078	59	30
B	1.7964	59	0
B			
B	1.7830	60	0.3
B			
B	1.7342	59	3

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 0.656419
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 0.2074
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	4.4275	118	M
B	2.5910	119	F

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 412.4289
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 5.1986
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	81.844	118	M
B	59.850	119	F

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 229 MSE= 2.104103
Critical Value of Studentized Range= 2.787
Minimum Significant Difference= 0.3713
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 118.4979

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	SEX
A	2.1087	119	F
A			
A	2.0492	118	M

Level of GRP	Level of SEX	N	T4 Mean	T4 SD	T3 Mean	T3 SD	TSH Mean	TSH SD
0	F	30	2.12633333	0.67732477	57.7696667	28.2040220	2.05400000	0.87308253
0	M	29	4.64103448	0.58348304	72.5465517	11.2281134	1.53000000	0.95718710
3	F	29	2.92413793	0.84142955	56.3503448	13.9917875	1.99000000	0.77299972
3	M	30	4.74400000	0.78984328	88.4523333	18.6341793	1.48700000	0.81502761
30	F	30	2.42133333	0.79200633	60.3733333	21.9703018	2.17366667	0.74373931
30	M	29	3.57758621	0.85996119	78.5703448	14.3634900	3.87068966	3.49483387
0.3	F	30	2.90333333	1.03876695	64.7890000	29.2769705	2.21333333	0.98685615
0.3	M	30	4.72600000	0.81742489	87.3893333	16.2571009	1.35266667	0.63824076

---------- SEX=F ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in by group = 119

SEX=F

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13.48606554	4.49535518	6.26	0.0006
Error	115	82.61141345	0.71836012		
Corrected Total	118	96.09747899			

	R-Square	C.V.	Root MSE		T4 Mean
	0.140337	32.71164	0.84756128		2.59100840

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	13.48606554	4.49535518	6.26	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	13.48606554	4.49535518	6.26	0.0006

-- SEX=F --

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1225.04156255	408.34718752	0.70	0.5559
Error	115	67405.32192989	586.13323417		
Corrected Total	118	68630.36349244			

	R-Square	C.V.	Root MSE		T3 Mean
	0.017850	40.45161	24.21018864		59.84974790

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	1225.04156255	408.34718752	0.70	0.5559

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	1225.04156255	408.34718752	0.70	0.5559

---------- SEX=F ----------

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.95342759	0.31780920	0.44	0.7250
Error	115	83.12068333	0.72278855		
Corrected Total	118	84.07411092			

	R-Square	C.V.	Root MSE	TSH Mean
	0.011340	40.31649	0.85016972	2.10873950

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	0.95342759	0.31780920	0.44	0.7250

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	0.95342759	0.31780920	0.44	0.7250

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 0.71836
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.573
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	2.9241	29	3
	A			
	A	2.9033	30	0.3
	A			
B	A	2.4213	30	30
B				
B		2.1263	30	0

--- SEX=F ---

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 586.1332
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 16.367
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	64.789	30	0.3
A			
A	60.373	30	30
A			
A	57.770	30	0
A			
A	56.350	29	3

-- SEX=F --

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 115 MSE= 0.722789
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.5747
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.74359

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	2.2133	30	0.3
A			
A	2.1737	30	30
A			
A	2.0540	30	0
A			
A	1.9900	29	3

-- SEX=M --

General Linear Models Procedure
Class Level Information

Class	Levels	Values
GRP	4	0 3 30 0.3

Number of observations in by group = 118

---------- SEX=M ----------

General Linear Models Procedure

Dependent Variable: T4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	27.94879729	9.31626576	15.69	0.0001
Error	114	67.70864000	0.59393544		
Corrected Total	117	95.65743729			

	R-Square	C.V.	Root MSE		T4 Mean
	0.292176	17.40665	0.77067207		4.42745763

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	27.94879729	9.31626576	15.69	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	27.94879729	9.31626576	15.69	0.0001

-- SEX=M --

General Linear Models Procedure

Dependent Variable: T3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	5050.24523342	1683.41507781	7.10	0.0002
Error	114	27040.90367506	237.20090943		
Corrected Total	117	32091.14890847			

	R-Square	C.V.	Root MSE		T3 Mean
	0.157372	18.81781	15.40132817		81.84440678

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	5050.24523342	1683.41507781	7.10	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	5050.24523342	1683.41507781	7.10	0.0002

----- SEX=M -----

General Linear Models Procedure

Dependent Variable: TSH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	128.07122848	42.69040949	12.21	0.0001
Error	114	398.71900287	3.49753511		
Corrected Total	117	526.79023136			

R-Square	C.V.	Root MSE	TSH Mean
0.243116	91.26175	1.87016981	2.04923729

Source	DF	Type I SS	Mean Square	F Value	Pr > F
GRP	3	128.07122848	42.69040949	12.21	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
GRP	3	128.07122848	42.69040949	12.21	0.0001

----- SEX=M -----

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T4

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 0.593935
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 0.5233
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	4.7440	30	3
A			
A	4.7260	30	0.3
A			
A	4.6410	29	0
B	3.5776	29	30

-- SEX=M --

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: T3

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 237.2009
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 10.457
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	GRP
	A	88.452	30	3
	A			
	A	87.389	30	0.3
	A			
B	A	78.570	29	30
B				
B		72.547	29	0

-- SEX=M --

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: TSH

NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 114 MSE= 3.497535
Critical Value of Studentized Range= 3.687
Minimum Significant Difference= 1.2698
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 29.49153

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	GRP
A	3.8707	29	30
B	1.5300	29	0
B			
B	1.4870	30	3
B			
B	1.3527	30	0.3

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

DATE: February 1, 1999

SUBJECT: Statistical analysis of ammonium perchlocate experiment

FROM: Dennis E. House *Dennis E. House*
NHEERL/BRSS/MD-55

TO: Andrew Geller
NHEERL/NTD/MD-74B

"Attached is the statistical analysis of the hormone data from the Argus Rat Developmental Neurotoxicology Study (Argus, 1998b). A memo from Argus Laboratories (RE: Argus Protocol #1416-001, 20 November 1998) contains thyroid hormone and thyrotrophin data from the Oral (Drinking Water) Two-Generation reproduction Study of ammonium perchlorate in the rat. Data were supplied on diskette in the form of ASCII text reports, one report for each gender/age group, and imported in ASCII form to SAS for further analysis.

The following is a statistical analysis of the thyroid and pituitary hormone data (T4, thyroxine; T3, triiodothyronine; TSH, thyroid stimulating hormone) found in that report. At the time of this analysis, data were available from both the F0 generation, females and males sacrificed at 5 and 6 months of age, respectively, and the F1 generation, one male and one female from each litter, sacrificed on postnatal day 21 (PND21). Males were sacrificed after 13 weeks of exposure, i.e., approximately 91 days. Females were sacrificed after 16 weeks, i.e. at weaning, approximately 120 days of exposure."

This report gives the results of some statistical analyses of the ammonium perchlocate experiment. The design of the experiment was to randomly assign rat parent pairs to one of four ammonium perchlocate dose groups. The doses were 0, .3, 3, and 30 (units unknown). Both parents were dosed 10 weeks before mating. Dosing of females continued through weaning or about age 21 days. One male and one female pup from each litter were sacrificed at age 21 days and TSH, T3, and T4 measurements were made.

The design of this experiment is a split-plot. The main plot treatment is the perchlocate dose which was applied to litters (since treatments were applied to the parents-mainly the mother) and the subplot "treatment" is gender. These designs are characterized by different mean square errors for evaluating different effects or classification variables in the experiment. Since three variables are measured on each pup, the proper analysis is a multivariate analysis of variance for a split-plot experiment. Essentially this is an analysis of the vector of three measurements from each pup.

The attached Table 1 gives the sample size, mean, and S.E. for each gender, dose, and variable combination. The means for the three variables are plotted in Figures 1 through 3. The multivariate analysis of variance results for the two main effects and the interaction effect are given in Table 2. All three effects are statistically significant (p<.05). The next step in the analysis is to do a univariate analysis of variance on each variable in order to understand the meaning of the significant multivariate effects. These latter analyses are for a spit-plot design. Since we are doing three analyses on one experiment, the Bonferroni adjustment to the p-values is made and are given under "Adjusted P" in each table. These adjusted p-values will be used to make conclusions from the experiment.

In the analysis of TSH, the dose by gender interaction is significant (p=.002) so a separate analysis of the dose effect only was done on each gender. The dose effect was not significant (p=.420) for females, but was significant (p<.001) for males. The conclusion for females is that there are no significant differences in TSH by dose. For males, Tukey's multiple comparison procedure was done on the dose means to determine which were different from each other. The conclusion for males is that dose 3 was significantly lower than doses 0 and 30 and no other differences are significant (p<.05).

The gender effect is the only significant one for T3 (p=.049). The mean of 108.4 (S.E.=1.5) for males is larger than the mean of 105.7 (S.E.=1.4) for females. The conclusion is that there are no dose effects on T3, but there is a small but significant gender effect.

No effect or interaction is significant for T4. The conclusion is that neither dose nor gender had a significant effect on T4.

Table 1
Means and S.E.s of each variable by gender and ammonium perchlorate dose

Gender	Ammonium perchlorate dose	TSH n	TSH Mean	TSH S.E.	T3 n	T3 Mean	T3 S.E.	T4 n	T4 Mean	T4 S.E.
Female	0	28	1.12	0.10	28	106	2.5	28	4.27	0.19
	.3	22	1.19	0.08	22	110	2.8	22	4.86	0.20
	3	25	1.14	0.07	25	109	2.7	25	4.32	0.16
	30	23	1.30	0.07	23	98	2.3	23	3.91	0.20
Male	0	27	1.24	0.09	27	106	1.9	27	4.40	0.20
	.3	21	0.94	0.07	21	111	3.6	21	4.61	0.21
	3	25	0.88	0.05	25	110	3.1	25	4.53	0.16
	30	23	1.27	0.08	23	107	3.3	23	4.53	0.23

Table 2
Multivariate analysis of variance of ammonium perchlorate data. Results for Wilks' Lambda statistic.

Source	D.F.	F	P
Dose	9, 226	2.29	.018
Gender	3, 89	5.69	.001
Dose x Gender	9, 217	3.67	<.001

Table 3
Analysis of variance of each variable in ammonium perchlorate experiment

Source	D.F.	Mean Square	F	P	Adjusted P
TSH					
Dose	3	.7510	3.13	.029	.087
Error 1	95	.2398			
Gender	1	.6243	10.85	.001	.004
Dose x Gender	3	.3745	6.51	<.001	.002
Error 2	91	.0576			
T3					
Dose	3	605.0	2.17	.097	.291
Error 1	95	279.0			
Gender	1	549.2	6.00	.016	.049
Dose x Gender	3	215.7	2.35	.077	.232
Error 2	91	91.6			
T4					
Dose	3	2.517	1.92	.132	.396
Error 1	95	1.314			
Gender	1	1.280	2.61	.109	.328
Dose x Gender	3	1.588	3.24	.026	.077
Error 2	91	.490			

Figure 1
TSH by dose and gender

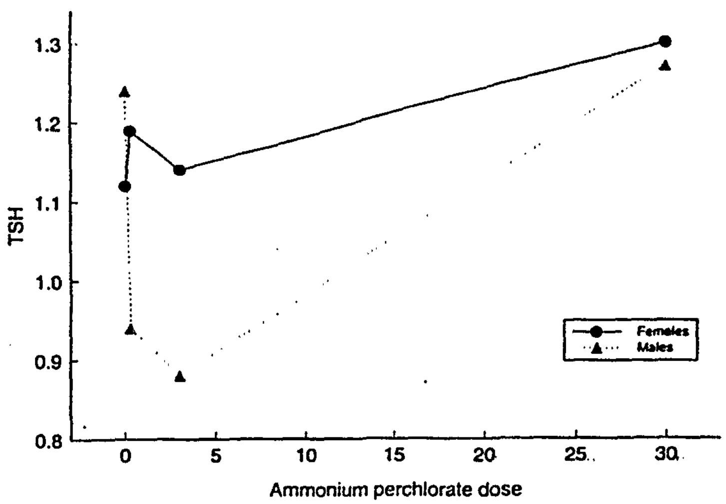


Figure 2
T3 by dose and gender

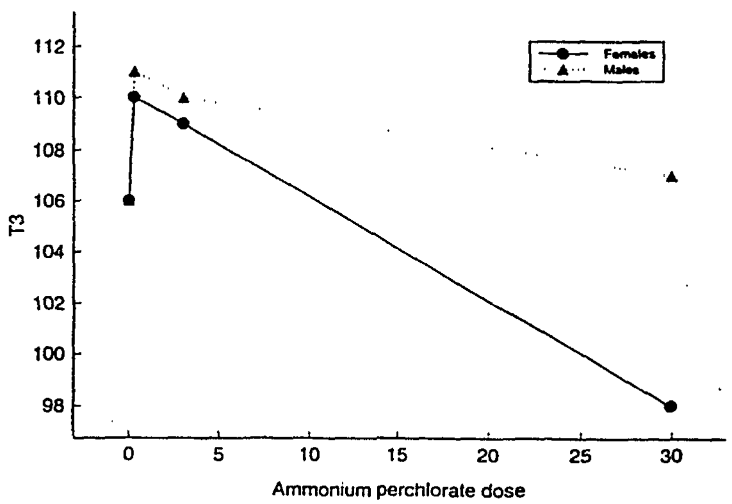
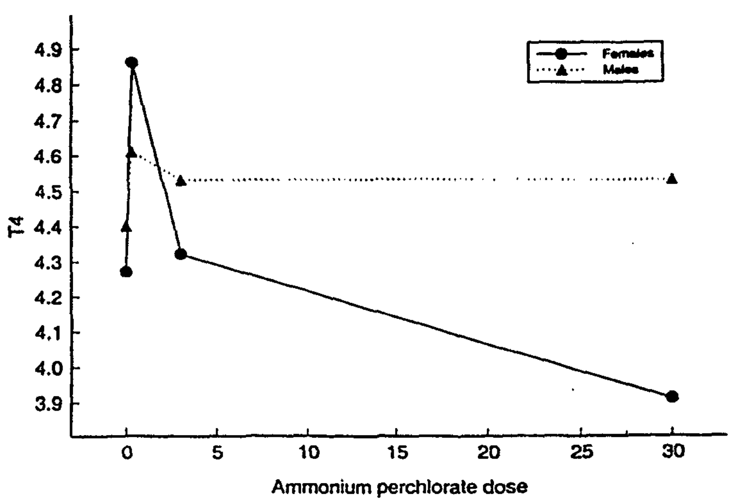


Figure 3
T4 by dose and gender



February 1, 1999 EPA Assessment Submission

Attachment #5
Analysis of Reproductive Parameters from the F1 Mating in Argus (1998b) 2-Generation Reproductive Study

A. Argus 1/15/99 Data Submission (York, 1999a)
B. EPA analysis (Clegg, 1999)

ATTENTION PANEL MEMBER(S):

ROCHELLE TYL

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

January 28, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Assimilation of F1 mating, estrous cyclicity and sperm measure results with P results

FROM: Eric D. Clegg, Ph.D. E. D. Clegg
National Center for Environmental Assessment (8623D)
Washington, DC

TO: Annie Jarabek
National Center for Environmental Assessment (MD-52)
Research Triangle Park, NC

I have reviewed the result tables on the F1 mating, estrous cyclicity and sperm measure results provided by Ray York of Argus Laboratories on January 15, 1999. The only statistically different result in the new data are in the fertility results where the control mating and pregnancy rates were significantly lower than the dosed groups. The values for the dosed groups were uniformly high. There was nothing remarkable in the results for the other parameters. The results with the P generation in mating and estrous cycle monitoring hinted at effects at 0.3 mg/kg, but those were not replicated with the F1 generation. Thyroid and ovarian weight data are not available yet for the F1. Thus, to this point, the F1 data are not supporting the existence of U-shaped dose-responses.

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

January 15, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached is a copy of the audited individual and summary tables with the F1 generation sperm and estrous cycling data you requested. Please remember, these data could still change based on the final audit of the other study data.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D15 (PAGE 1): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS EXAMINED	N	30	30	30	27a
INCLUDED IN ANALYSES	N	29b	30	29b	27
NUMBER MOTILE	MEAN±S.D.	420.6 ± 158.4	400.4 ± 163.2	397.9 ± 186.0	449.0 ± 145.0
MOTILE PERCENT	MEAN±S.D.	77.2 ± 7.8	76.9 ± 8.1	76.4 ± 7.2	80.6 ± 5.7
STATIC COUNT (NONMOTILE)	MEAN±S.D.	116.1 ± 38.6	110.6 ± 39.7	114.6 ± 48.3	107.1 ± 45.4
TOTAL COUNT	MEAN±S.D.	536.6 ± 171.6	511.0 ± 181.3	512.5 ± 212.9	556.1 ± 170.6
SPERM COUNT	MEAN±S.D.	186.9 ± 59.7	200.9 ± 73.1	181.3 ± 53.9	179.0 ± 61.8
SPERM CONCENTRATION	MEAN±S.D	10.9 ± 2.4	11.8 ± [illegible]	10.6 ± 3.[illegible]	10.4 ± 3.6
SPERM DENSITY	MEAN±S.D.	1543.6 ± 520.8	1571.6 ± 536.1	1461.2 ± 438.8	1372.6 ± 444.6
SPERMATID COUNT	MEAN±S.D.	36.6 ± 15.5	35.6 ± 14.0	33.4 ± 9.4	29.6 ± 12.1
SPERMATID CONCENTRATION	MEAN±S.D.	2.1 ± 0.9	2.1 ± 0.8	1.9 ± 0.5	1.7 ± 0.8
SPERMATID DENSITY	MEAN±S.D.	125.0 ± 44.4	117.2 ± 46.2	109.3 ± 28.6	97.6 ± 41.2

a. Excludes values for rats that were found dead.

b. Excludes values for rats that had abnormal epididymides and testes.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D16 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS EXAMINED	N	30	30	30	27a
INCLUDED IN ANALYSES	N	29b	30	29b	27
NORMAL	MEAN±S.D.	189.5± 6.3	188.8± 4.9	190.1± 4.9	188.1± 5.4
PERCENT ABNORMAL	MEAN±S.D.	5.4± 3.1	5.6± 2.5	4.9± 2.4	5.9± 2.7
NO HOOK	MEAN±S.D.	0.2± 0.5	0.2± 0.6	0.1± 0.2	0.1± 0.4
EXCESSIVE HOOK	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.2
AMORPHOUS	MEAN±S.D.	0.0± 0.0	0.1± 0.2	0.0± 0.0	0.0± 0.2
PIN HEAD	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.2
DETACHED HEAD	MEAN±S.D.	7.9± 5.1	7.7± 4.1	6.9± 4.0	8.1± 5.0
NO HEAD	MEAN±S.D.	2.2± 1.6	2.6± 2.0	2.4± 1.4	2.8± 1.9
BANANA	MEAN±S.D.	0.0± 0.2	0.1± 0.2	0.1± 0.4	0.2± 0.8
COILED FLAGELLUM	MEAN±S.D.	0.0± 0.2	0.1± 0.2	0.0± 0.2	0.0± 0.0
BENT FLAGELLUM	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.2	0.0± 0.2
BENT FLAGELLUM TIP	MEAN±S.D.	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
BROKEN FLAGELLUM	MEAN±S.D.	0.5± 0.8	0.5± 0.8	0.3± 0.7	0.4± 0.6

a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal epididymides and testes.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 1): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 1 0 (CARRIER) MG/KG/DAY

RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7001	192	74	69	261	163	9.4	1399.1	43	2.5	163.5
7002	438	76	142	580	96	5.6	826.5	48	2.8	182.1
7003	588	90	64	652	182	10.5	1339.6	26	1.5	90.3
7004d	0	0	6	6	2	0.1	41.3	16	0.9	279.7
7005	360	76	113	473	237	13.7	1722.5	34	2.0	120.9
7006	178	56	142	320	146	8.4	1422.0	26	1.5	100.5
7007	281	75	92	373	247	14.3	2205.2	33	1.9	107.4
7008	447	85	77	524	254	14.7	1800.9	27	1.6	97.2
7009	211	74	73	284	243	14.1	2466.4	33	1.9	112.8
7010	669	83	133	802	183	10.6	1470.5	29	1.7	97.4
7011	235	81	54	289	165	9.5	1391.5	29	1.7	107.8
7012	346	70	145	491	234	13.5	2338.1	35	2.0	110.2
7013	657	85	116	773	341	19.7	2644.5	4	0.2	12.6
5650	711	80	180	891	169	9.8	1433.6	33	1.9	117.1
7015	559	85	100	659	157	9.1	1316.4	21	1.2	77.0
7016	274	69	122	396	310	17.9	2591.7	26	1.5	95.2
7017	654	83	131	785	195	11.3	1500.2	37	2.1	135.5
7018	351	84	66	417	178	10.3	1418.5	33	1.9	122.7
7019	332	76	107	439	225	13.0	2148.1	27	1.6	115.0
7020	326	69	149	475	144	8.3	[illegible]	59	[illegible]	[illegible]
7021	626	86	98	724	155	9.0	1042.7	20	1.2	69.7
7022	456	74	161	617	107	6.2	834.3	44	2.5	159.3
7023	464	87	71	535	190	11.0	1473.5	46	2.7	142.0
7024	625	79	166	791	158	9.1	1151.3	78	4.5	207.4
7025	347	73	128	475	125	7.2	1092.4	35	2.0	130.6
7026	289	78	82	371	169	9.8	1384.9	26	1.5	89.9
7027	490	85	85	575	248	14.3	1897.9	44	2.5	142.5
7028	452	73	169	621	187	10.8	1494.3	66	3.8	209.9
7029	309	62	193	502	101	5.8	936.4	33	1.9	128.2
7030	329	70	138	467	111	6.4	991.0	67	3.9	212.9

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.9×10^{-6}), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Rat 7004 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 2): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 2			9.3 MG/KG/DAY							
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7031	162	72	62	224	176	10.2	1414.2	15	0.9	45.6
7032	455	81	105	560	171	9.9	1242.8	38	2.2	110.4
7033	192	71	79	271	199	11.5	1560.0	23	1.3	80.1
7034	475	71	194	669	131	7.6	1013.2	27	1.6	86.1
7035	326	78	92	418	226	13.1	1594.5	34	2.0	101.7
7036	314	80	79	393	224	13.0	1810.0	52	3.0	159.2
7037	311	78	87	398	286	16.5	2154.5	40	2.3	128.8
7038	537	77	161	698	256	14.8	2208.0	29	1.7	99.3
7039	277	68	129	406	329	19.0	2524.4	33	1.9	121.4
7040	438	80	108	546	216	12.5	1899.2	39	2.3	120.4
7041	212	70	89	301	199	11.5	1683.2	15	0.9	52.8
7042	436	90	48	484	292	16.9	2513.9	8	0.5	32.3
7043	423	78	117	540	265	15.3	2100.2	41	2.4	121.5
7044	523	92	48	571	205	11.9	1382.3	39	2.3	137.2
7045	620	82	133	753	179	10.4	1341.4	17	1.0	58.5
7046	236	63	136	372	293	17.0	2278.4	21	1.2	67.4
7047	339	85	58	397	325	18.8	2254.5	15	0.9	45.9
7048	222	79	59	281	84	4.9	843.7	39	2.3	144.7
7049	568	83	114	682	113	6.5	1089.6	44	2.5	180.0
7050	175	60	115	290	256	18.4	2005.3	51	3.5	189.2
7051	592	77	174	766	126	7.3	1088.0	38	2.2	130.1
7052	785	84	147	932	176	10.2	1308.8	39	2.3	128.8
7053	316	74	110	426	292	16.9	1968.9	54	3.1	182.5
7054	167	61	107	274	257	14.9	1891.7	53	3.1	152.7
7055	400	68	192	592	91	5.3	849.2	44	2.5	147.8
7056	517	80	131	648	121	7.0	1035.6	43	2.5	167.9
7057	419	85	72	491	100	5.8	747.5	49	2.8	162.5
7058	687	88	98	785	201	11.6	1498.5	25	1.4	73.6
7059	542	81	129	671	127	7.3	959.2	32	1.9	98.9
7060	345	70	146	491	112	6.5	892.5	61	3.5	192.0

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6}), multiplying by 2 (dilution factor) and multiplying by 10^{-4} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D25 (PAGE 3): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 3				3.0 MG/KG/DAY						
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7061	336	74	117	453	190	11.0	1531.0	32	1.9	96.9
7062	257	74	92	349	159	9.2	1390.0	26	1.5	83.3
7063	212	64	117	329	142	8.2	1229.8	18	1.0	65.8
7064	169	68	80	249	196	11.3	1723.3	43	2.5	141.8
7065	246	77	75	321	202	11.7	1240.6	38	2.2	105.9
7066	311	74	108	419	177	10.2	1296.2	24	1.4	90.3
7067	274	64	153	427	306	17.7	2445.2	24	1.4	77.6
7068	215	77	66	281	262	15.2	2468.7	27	1.6	100.8
7069	622	80	160	782	195	11.3	1532.8	34	2.0	118.2
7070d	0	0	8	8	6	0.3	92.8	0	0.0	0.0
7071	331	76	103	434	168	9.7	1717.2	24	1.4	83.3
7072	162	72	64	226	286	16.5	2224.0	38	2.2	114.5
7073	201	64	113	314	89	5.1	770.8	33	1.9	111.8
7074	558	82	125	683	168	9.7	1327.8	31	1.8	93.4
7075	834	91	83	917	138	8.0	1078.9	14	0.8	42.8
7076	274	87	42	316	164	9.5	1379.1	37	2.1	115.1
7077	338	80	86	424	144	8.3	980.1	45	2.6	148.1
7078	699	92	58	757	127	7.3	1043.7	48	2.8	139.1
7079	312	81	71	383	146	8.4	1456.3	32	1.9	120.6
7080	398	71	163	561	169	9.8	1800.8	47	2.7	149.2
7081	446	77	130	576	157	9.1	1170.5	28	1.6	96.9
7082	461	85	84	545	147	10.6	1436.6	27	1.6	95.6
7083	285	71	115	400	196	11.3	1633.9	42	2.4	160.3
7084	335	79	90	425	151	8.7	1456.0	29	1.7	103.0
7085	638	73	231	869	130	7.5	924.0	34	2.0	108.5
7086	408	81	95	503	190	11.0	1380.9	39	2.3	128.3
7087	346	78	100	446	239	13.8	1942.0	57	3.3	176.4
7088	425	68	200	625	155	9.0	1088.3	31	1.8	96.3
7089	704	79	185	889	315	18.2	2139.0	28	1.6	97.5
7090	742	77	217	959	150	8.7	957.9	40	2.3	109.3

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-4}), multiplying by 2 (dilution factor) and multiplying by 10^{-4} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Rat 7070 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D26 (PAGE 4): CAUDA EPIDIDYMAL SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - F1 GENERATION MALE RATS

DOSAGE GROUP 4			30.0 MG/KG/DAY							
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM CONCENTRATION	SPERM DENSITY c	SPERMATID COUNT	SPERMATID CONCENTRATION	SPERMATID DENSITY
7091	355	70	153	508	124	7.2	1087.0	25	1.4	91.4
7092	245	76	76	321	295	17.1	2056.3	33	1.9	95.2
7093	540	72	210	750	190	11.0	1557.0	51	3.7	194.7
7094	296	84	58	354	271	15.7	1819.9	28	1.6	84.1
7095	425	78	120	545	190	11.0	1548.2	27	1.6	89.2
7096	249	79	65	314	224	13.0	1670.0	40	2.3	134.0
7097	471	82	102	573	213	12.3	1760.4	22	1.3	84.2
7098	647	81	154	801	120	6.9	1084.8	18	1.0	65.9
7099	467	89	56	523	135	7.8	1259.7	25	1.4	82.5
7100	333	86	56	389	245	14.2	2109.3	16	0.9	59.0
7101	397	85	69	466	215	12.4	1671.9	18	1.0	62.5
7102	249	80	62	311	273	15.8	2131.4	18	1.0	54.8
7103	431	78	125	556	228	13.2	1653.0	14	0.8	39.0
7104	638	88	83	721	132	7.6	1051.9	15	0.9	53.6
7105	562	83	114	676	103	6.0	721.4	18	1.0	41.9
7106	495	88	69	564	80	4.6	628.9	31	1.8	100.9
7107	212	75	70	282	136	7.9	936.7	4	0.2	11.9
7108	437	78	126	563	98	5.7	869.6	38	2.2	128.5
7109	444	68	207	651	140	8.1	1121.8	38	2.2	124.8
7110	836	85	144	980	283	16.4	1944.5	43	2.5	129.9
7111	FOUND DEAD ON DAY 131 POSTWEANING									
7112	605	81	146	751	159	9.2	1054.9	37	2.1	129.7
7113	FOUND DEAD ON DAY 95 POSTWEANING									
7114	537	75	182	719	152	8.8	1289.4	48	2.8	181.0
7115	FOUND DEAD ON DAY 82 POSTWEANING									
7116	467	84	89	556	139	8.0	1104.6	26	1.5	94.2
7117	293	75	100	393	195	11.3	1598.0	42	2.4	131.8
7118	552	87	83	635	225	13.0	1575.9	40	2.3	107.3
7119	508	87	79	587	114	6.6	774.1	44	2.5	127.7
7120	433	82	93	526	154	8.9	976.9	39	2.3	115.8

a. Sum of number motile and static count.

b. Sperm count used in the calculation of sperm density.

c. The sperm density is calculated by dividing the sperm count by the volume in the image area (34.3×10^{-4}), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) is multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table D24 for the weight of the left cauda epididymis, rounded to 3 decimal places) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

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TABLE D27 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCES-SIVE HOOK	AMOR-PHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	BROKEN FLAGEL-LUM	PERCENT ABNORMAL
DOSAGE GROUP 1						0 (CARRIER) MG/KG/DAY							
7001	194	0	0	0	0	3	2	0	0	0	0	1	3.0
7002	168	2	0	0	0	25	3	0	0	0	0	2	16.0
7003	187	0	0	0	0	8	3	0	0	0	0	2	6.5
7004a	9	0	0	0	0	1	8	0	0	0	0	0	50.0
7005	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7006	195	0	0	0	0	4	1	0	0	0	0	0	2.0
7007	192	0	0	0	0	4	3	0	0	0	0	0	2.5
7008	192	0	0	0	0	7	1	0	0	0	0	1	4.0
7009	181	1	0	0	0	10	7	0	0	0	0	0	4.0
7010	193	0	0	0	0	7	0	0	0	0	0	1	9.5
7011	177	0	0	0	0	20	3	0	0	0	0	0	3.5
7012	188	0	0	0	0	9	3	0	0	0	0	0	11.5
7013	195	0	0	0	0	3	2	0	0	0	0	0	6.0
5450	188	1	0	0	0	9	1	0	1	0	0	0	2.5
7015	192	1	0	0	0	4	3	0	0	0	0	0	6.0
7016	195	0	0	0	0	1	2	1	0	0	0	1	4.0
7017	187	1	0	0	0	8	3	0	0	0	0	1	2.5
7018	188	0	0	0	0	8	4	0	0	0	0	0	6.5
7019	186	1	0	0	0	7	3	0	0	0	0	3	6.0
7020	192	0	0	0	0	4	3	0	0	0	0	1	7.0
7021	190	0	0	0	0	9	1	0	0	0	0	0	4.0
7022	196	0	0	0	0	4	0	0	0	0	0	0	5.0
7023	196	0	0	0	0	4	0	0	0	0	0	0	2.0
7024	184	0	0	0	0	14	2	0	0	0	0	0	2.0
7025	189	0	0	0	0	9	2	0	0	0	0	0	8.0
7026	191	0	0	0	0	9	0	0	0	0	0	0	5.5
7027	184	0	0	0	0	12	4	0	0	0	0	0	4.5
7028	192	0	0	0	0	4	4	0	0	0	0	0	8.0
7029	190	0	0	0	0	9	1	0	0	0	0	0	4.0
7030	197	0	0	0	0	9	3	0	0	0	0	1	5.0

a. Rat 7004 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 2): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - F1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCESSIVE HOOK	AMORPHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	BROKEN FLAGELLUM	PERCENT ABNORMAL
DOSAGE GROUP 2						0.3 MG/KG/DAY							
7031	190	0	0	0	0	8	1	0	0	0	0	1	5.0
7032	183	0	0	0	0	14	2	0	0	0	0	1	8.5
7033	194	0	0	0	0	5	1	0	0	0	0	0	3.0
7034	191	1	0	0	0	3	5	0	0	0	0	0	4.5
7035	193	0	0	1	0	4	1	0	0	0	0	1	3.5
7036	190	0	0	0	0	6	3	0	1	0	0	0	5.0
7037	181	0	0	0	0	9	7	1	0	0	0	2	9.5
7038	192	0	0	0	0	5	3	0	0	0	0	0	4.0
7039	187	0	0	0	0	8	3	0	0	0	0	2	6.5
7040	189	0	0	0	0	9	1	0	1	0	0	0	5.5
7041	185	0	0	0	0	9	3	0	0	0	0	3	7.5
7042	196	0	0	0	0	3	1	0	0	0	0	0	2.0
7043	189	0	0	0	0	4	7	0	0	0	0	0	5.5
7044	190	0	0	0	0	8	1	0	0	0	0	1	5.0
7045	195	0	0	0	0	4	0	1	0	0	0	0	2.5
7046	186	2	0	0	0	7	4	0	0	0	0	1	7.0
7047	186	2	0	1	0	7	2	0	0	0	0	0	6.0
7048	182	0	0	0	0	13	5	0	0	0	0	0	9.0
7049	188	0	0	0	0	10	2	0	0	0	0	0	6.0
7050	189	1	0	0	0	8	2	0	0	0	0	0	5.5
7051	194	0	0	0	0	4	2	0	0	0	0	0	3.0
7052	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7053	182	0	0	0	0	18	0	0	0	0	0	0	9.0
7054	193	0	0	0	0	5	1	0	0	0	0	1	3.5
7055	189	0	0	0	0	9	1	0	0	0	0	1	5.5
7056	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7057	174	0	0	0	0	18	7	0	0	0	0	1	13.0
7058	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7059	185	0	0	0	0	13	2	0	0	0	0	0	7.5
7060	195	0	0	0	0	3	1	0	0	0	0	1	2.5

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 3): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - P1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCES-SIVE HOOK	AMOR-PHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	BROKEN FLAGEL-LUM	PERCENT ABNORMAL
DOSAGE GROUP 3						3.0 MG/KG/DAY							
7061	194	0	0	0	0	4	2	0	0	0	0	0	3.0
7062	190	1	0	0	0	2	3	0	1	0	0	3	5.0
7063	185	0	0	0	0	10	5	0	0	0	0	0	7.5
7064	188	0	0	0	0	8	3	0	0	0	0	1	6.0
7065	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7066	193	0	0	0	0	4	2	1	0	0	0	0	3.5
7067	195	0	0	0	0	3	2	0	0	0	0	0	2.5
7068	191	0	0	0	0	5	4	0	0	0	0	0	4.5
7069	193	0	0	0	0	6	1	0	0	0	0	0	3.5
7070a	11	0	0	0	0	2	8	0	0	0	0	0	47.6
7071	191	0	0	0	0	6	2	0	0	1	0	0	4.5
7072	186	1	0	0	0	11	2	0	0	0	0	0	7.0
7073	195	0	0	0	0	4	1	0	0	0	0	0	2.5
7074	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7075	197	0	0	0	0	2	1	0	0	0	0	0	1.5
7076	191	0	0	0	0	7	2	0	0	0	0	0	4.5
7077	173	0	0	0	0	20	7	0	0	0	0	0	13.5
7078	193	0	0	0	0	7	0	0	0	0	0	0	3.5
7079	188	0	0	0	0	10	2	0	0	0	0	0	6.0
7080	188	0	0	0	0	9	2	1	0	0	0	0	6.0
7081	185	0	0	0	0	11	3	0	0	0	0	1	7.5
7082	196	0	0	0	0	2	2	0	0	0	0	0	2.0
7083	191	0	0	0	0	6	3	0	0	0	0	0	4.5
7084	195	0	0	0	0	3	2	0	0	0	0	0	2.5
7085	190	0	0	0	0	8	1	1	0	0	0	0	5.0
7086	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7087	190	0	0	0	0	5	3	0	0	0	0	2	5.0
7088	185	0	0	0	0	14	1	0	0	0	0	0	7.5
7089	184	0	0	0	0	10	4	1	0	0	0	1	8.0
7090	195	0	0	0	0	3	2	0	0	0	0	0	2.5

a. Rat 7070 had small and flaccid epididymides and testes; values excluded from group averages and statistical analyses.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D27 (PAGE 4): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - P1 GENERATION MALE RATS

ANIMAL NUMBER	NORMAL	NO HOOK	EXCES-SIVE HOOK	AMOR-PHOUS	PIN HEAD	DETACHED HEAD	NO HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	BROKEN FLAGEL-LUM	PERCENT ABNORMAL
DOSAGE GROUP 4						30.0 MG/KG/DAY							
7091	190	0	0	0	0	8	2	0	0	0	0	0	5.0
7092	174	0	0	0	0	22	3	0	0	0	0	1	13.0
7093	182	0	0	0	0	17	1	0	0	0	0	0	9.0
7094	194	0	0	0	0	6	0	0	0	0	0	0	3.0
7095	189	0	0	0	0	7	4	0	0	0	0	0	5.5
7096	190	0	0	1	0	4	4	0	0	0	0	1	5.0
7097	184	0	0	0	0	11	3	0	0	0	0	2	8.0
7098	193	0	0	0	1	6	0	0	0	0	0	0	3.5
7099	192	0	0	0	0	4	4	0	0	0	0	0	4.0
7100	192	0	0	0	0	7	1	0	0	0	0	0	4.0
7101	188	2	0	0	0	0	6	4	0	0	0	0	6.0
7102	196	0	0	0	0	1	3	0	0	0	0	0	2.0
7103	188	0	0	0	0	8	4	0	0	0	0	0	6.0
7104	181	0	0	0	0	13	6	0	0	0	0	0	9.5
7105	183	0	0	0	0	11	5	0	0	0	0	1	8.5
7106	194	0	0	0	0	2	3	0	0	0	0	1	3.0
7107	190	0	0	0	0	7	2	0	0	0	0	1	5.0
7108	188	0	0	0	0	5	6	0	0	0	0	1	6.0
7109	181	0	0	0	0	10	5	0	0	0	0	1	8.1
7110	188	1	0	0	0	8	3	0	0	0	0	0	6.0
7111	FOUND DEAD ON DAY 131 POSTWEANING												
7112	192	0	0	0	0	6	2	0	0	0	0	0	4.0
7113	FOUND DEAD ON DAY 95 POSTWEANING												
7114	193	0	0	0	0	5	1	0	0	0	0	1	3.5
7115	FOUND DEAD ON DAY 82 POSTWEANING												
7116	188	0	0	0	0	8	3	0	0	0	0	1	6.0
7117	195	0	0	0	0	5	0	0	0	0	0	0	2.5
7118	191	0	0	0	0	8	0	1	0	0	0	0	4.5
7119	181	0	0	0	0	17	2	0	0	0	0	0	9.5
7120	181	0	1	0	0	12	4	0	0	1	0	1	9.5

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E22 (PAGE 1): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
PRECOHABITATION ESTROUS CYCLING					
RATS EVALUATED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	29a	30	30
ESTROUS STAGES/ 14 DAYS	MEAN±S.D.	5.0 ± 0.8	4.8 ± 0.8	4.9 ± 0.7	4.9 ± 1.0
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF DIESTRUS	N	3	3	0	3
RATS WITH 6 OR MORE CONSECUTIVE DAYS OF ESTRUS	N	0	0	0	0

a. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E22 (PAGE 2): ESTROUS CYCLING, MATING AND FERTILITY - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS IN COHABITATION	N	30	29a	30	30
DAYS IN COHABITATION b	MEAN±S.D	2.9 ± 2.8	3.2 ± 2.6	2.9 ± 1.6	2.4 ± 1.2 [29]c
RATS THAT MATED	N(%)	29(96.7)	28(96.6)	30(100.0)	30(100.0)
FERTILITY INDEX d	N/N (%)	21/29 (72.4)	27/28** (96.4)	28/30** (93.3)	27/30** (90.0)
RATS WITH CONFIRMED MATING DATES	N	29	28	30	29c
RATS MATING c,e DAYS 1- 7	N(%)	28(96.6)	28(100.0)	30(100.0)	29(96.7)
DAYS 8-14	N(%)	1(3.4)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N (%)	21/30 (70.0)	27/29** (93.1)	28/30** (93.3)	27/30** (90.0)

[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).
b. Restricted to rats with a confirmed mating date and rats that did not mate.
c. Excludes values for dam 7307, which was cohabited with a second male rat.
d. Number of pregnancies/number of rats that mated.
e. Restricted to rats with a confirmed mating date.
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 1): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 1		0 (CARRIER) MG/KG/DAY			
7201	5	3	7216	5	1
7202	4a	1	7217	5	1
7203	6	4	7218	5	2
7204	5	1	7219	5	1
7205	3a	3	7220	4	2
7206	6	4	7221	4	1
7207	5	5	7222	5	2
7208	5	9	7223	5	1
[illegible]	4	2	7224	5	3
7210	4a	3	7225	6	1
7211	3	3	7226	6	1
7212	5	3	7227	5	3
7213	5	3	7228	6	4
7214	6	1	7229	5	3
7215	6	5	7230	6	14b

a. Six or more consecutive days of diestrus were observed.
b. Mating not confirmed.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 2): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 2			0.3 MG/KG/DAY		
7231	3a	6	7246	5	1
7232	5	6	7247	5	2
7233	6	4	7248	5	2
7234	5	2	7249	5	1
7235	5	2	7250	3a	3
7236	5	3	7251	4	3
7237	3	1	7252	5	4
7238	5	1	7253b		
7239	5	3	7254	5	1
7240	5	3	7255	3a	7
7241	5	2	7256	5	3
7242	6	4	7257	5	2
7243	5	14c	7258	5	4
7244	5	3	7259	5	4
7245	5	3	7260	5	2

a. Six or more consecutive days of diestrus were observed.
b. Rat 7253 was moribund sacrificed on day 62 postweaning (day 3 of estrous cycling).
c. Mating not confirmed.

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TABLE E44 (PAGE 3): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 3			3.0 MG/KG/DAY		
7261	5	1	7276	5	2
7262	6	4	7277	5	1
7263	5	7	7278	5	2
7264	5	4	7279	5	1
7265	4	2	7280	5	6
7266	6	1	7281	4	6
7267	4	2	7282	5	3
7268	5	1	7283	5	3
7269	3	2	7284	5	2
7270	5	3	7285	5	2
7271	5	3	7286	6	4
7272	6	4	7287	5	2
7273	3	5	7288	5	3
7274	5	3	7289	5	3
7275	5	1	7290	5	3

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E44 (PAGE 4): ESTROUS CYCLING AND DAYS IN COHABITATION - INDIVIDUAL DATA - F1 GENERATION FEMALE RATS

RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION	RAT #	PRECOHABITATION ESTROUS STAGES/ 21 DAYS	DAYS IN COHABITATION
DOSAGE GROUP 4			30.0 MG/KG/DAY		
7291	6	1	7306	6	4
7292	4a	3	7307	6	8b
7293	3	1	7308	5	4
7294	6	4	7309	6	4
7295	4	1	7310	5	2
7296	6	2	7311	5	2
7297	6	4	7312	3	3
7298	6	2	7313	4	1
7299	4a	2	7314	4	1
7300	4	2	7315	5	1
7301	5	6	7316	5	3
7302	5	3	7317	5	2
7303	4	3	7318	5	2
7304	3a	3	7319	4	2
7305	5	2	7320	7	1

a. Six or more consecutive days of diestrus were observed.
c. Dam 7307 was cohabited with a second male rat; values excluded from group averages and statistical analyses.

February 1, 1999 EPA Assessment Submission

Attachment #6
Sheep Red Blood Cell (SRBC) Assay in 90-day Studies

A. Keil 1/23/99 Data Submission
B. EPA analysis (Smialowicz, 1999)

ATTENTION PANEL MEMBER(S):

KIMBER WHITE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Experimental Toxicology Division
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

DATE: January 28, 1999

FROM: Ralph J. Smialowicz (MD-92) R.J. Smialowicz

TO: Annie Jarabek (MD-52)
National Center for Environmental Assessment

SUBJECT: Review of 90-Day Ammonium Perchlorate Exposure on the Antibody Response to SRBC in Mice

As indicated in the external review draft of the NCEA document entitled *Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information*, an evaluation of the potential effects of ammonium perchlorate on humoral immunity was not performed as part of the original immunotoxicity testing protocol. This raised concern that a significant component of the immune system was not assessed in perchlorate-exposed animals. Consequently, the sponsor and contract laboratory agreed to perform 14-day and 90-day studies in which the antibody response to sheep red blood cells (SRBC) would be determined.

Results of a 90-day study were received on January 23,1999. In this study, B6C3F1 female mice, 12 mice per group, were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. Mice where immunized intravenously with SRBC on day 75. Serum was collected on day 79 (4 days post-immunization) and on day 90 (15 days post-immunization), and the SRBC-specific IgM and IgG antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA) "based on a protocol provided by L. Temple of the Medical College of Virginia". Analysis of the ELISA data, which was expressed as the O.D. 50, indicated that neither the IgM nor IgG titers were affected by ammonium perchlorate exposure. In the report, the contract laboratory indicated limitations which were the following: 1) a kinetic study to determine the day of peak levels of IgM and IgG was not performed; and 2) since specialized software (e.g., Softmax®) was not available, serum antibody titers were calculated as the O.D. 50 or midpoint "as described by a SOP provided by L. Temple", rather than the conventional "titer to achieve 0.5 O.D.".

The results of a 14-day exposure study on SRBC-specific antibody responses in mice is expected on February 3, 1999. In addition, because of concern expressed in the external review draft about the infectivity data (i.e., *L. monocytogenes* challenge model) additional studies are currently in progress. The expected due date for the report of these data is June 1, 1999.

SRBC Specific Serum IgM or IgG Determination after Exposure to Ammonium Perchlorate for 90 Days

Submitted by Deborah Keil, PhD
Medical University of South Carolina
January 23, 1999

Animals and Ammonium Perchlorate Exposure: B6C3F1 female mice aged 8-10 weeks were exposed to ammonium perchlorate (AP) (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. A total of 60 mice with 12 animals per treatment group were used to determine specific IgM and IgG levels after immunization with sRBC. Animals were housed in an AAALAC accredited facility and provided water (with and without AP) and mouse chow ad libidum.

Immunization: Mice were immunized with sheep red blood cells (sRBC) (1×10^8 total cells) by intravenous tail injection on day 75. Serum was collected on day 79 (4 days post challenge) and day 90 (15 days post challenge) to determine specific IgM or IgG sRBC antibody levels, respectively. A semi-quantitative ELISA detected levels of specific IgM or IgG sRBC antibody in serially diluted serum (1:20, 1:40, 1:80, 1:160, 1:320). A SOP based on a protocol provided by L. Temple of the Medical College of Virginia was used.

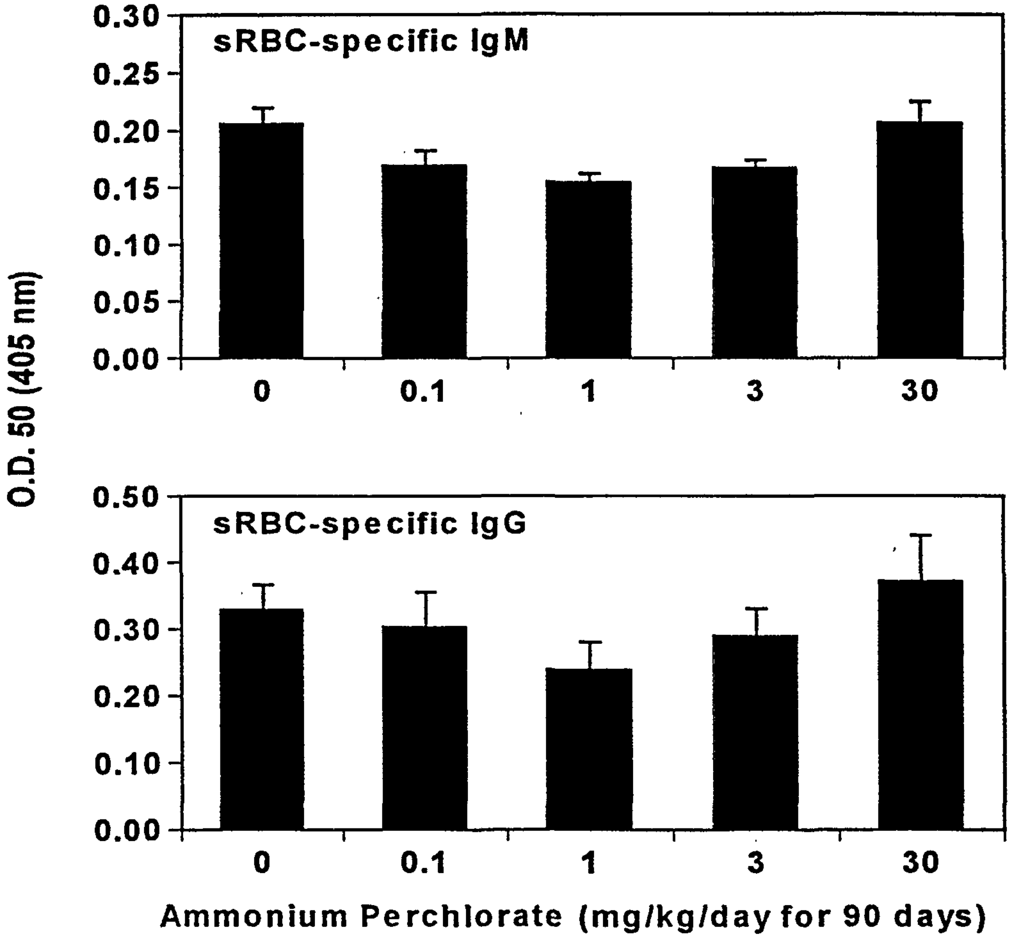
Optimization of the ELISA: Optimization of the ELISA was performed prior to testing the serum samples to establish the appropriate titer of sRBC membrane coating antigen (1μg/ml) and the secondary antibody dilution (1: 5,000 for IgM and 1:7,500 for IgG). In addition, pooled serum samples from controls were used in the optimization. Controls for non-specific binding were included and were less than 0.070 O.D. (405 nm) in both the optimization and testing ELISAs.

Data Analysis:
Analysis of sRBC specific IgG serum titers were analyzed as described in a SOP provided by Louise Temple of the Medical College of Virginia. The average absorbance unit values of the replicates for each dilution of the test serum were calculated. Background in the ELISA was subtracted from these values. Five consecutive average absorbance values versus log base 2 of the dilution of the serum were plotted. The best-fit linear line was calculated in an Excel spreadsheet by determining the value for the slope and intercept. Log base 2 of the titer was considered the independent variable and O.D. was considered the dependent variable. In this experiment, the absorbance at the mid-point of the 5 serial dilutions was 1:80 (log base 2 (80) = 6.3219). Using the equation for the best-fit line, the O.D. 50 (absorbance at mid-point 1:80) was calculated for each animal.

Results:
No significant differences were observed in any of the AP treatment groups as compared to controls for specific IgM or IgG levels after immunization with sRBC. This was determined by using the calculated O.D.50 for each sample and performing an analysis of variance with Tukey's pairwise comparisons ($p<0.05$). Refer to graphs and statistical analysis that have been included in this report.

Limitations: A time course to determine the peak levels of IgM or IgG after sRBC immunization in B6C3F1 female mice was not performed in this study. However, bleeding times (day 4 for IgM and day 15 for IgG) have been previously used and reported in the literature (Holsapple, et al, 1984).
In addition, these data may be analyzed by additional methods to include expression of the "serum titer to achieve 0.5 O.D." At this time, the data manipulation involved to determine the "serum titer to achiev 0.5 O.D." has been laborious and time-consuming, particularly when specialized software (i.e., Softmax) is not available to produce specialized graphs and corresponding equations for each of the 120 samples. Consequently, I have submitted the calculated O.D. 50 as described by a SOP provided by L. Temple.

Serum IgM or IgG Levels after sRBC Challenge During a 90-Day Exposure to Ammonium Perchlorate



Adult B6C3F1 female mice were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 90 days. On day 75, animals were immunized by i.v. tail injection with sRBC (1×10^8 cells). Following the immunization, animals were bled on day 79 (4 days post challenge) and day 90 (15 days post challenge) to obtain serum for detection of specific IgM or IgG respectively. Detection of specific IgM or IgG was performed using an ELISA based on a protocol provided by L. Temple at the Medical College of Virginia. The O.D. 50 was determined for both IgM and IgG. Each of the above graphs represent the means and standard errors of a total of 59 mice. No significant differences were observed in any of the treatment groups as compared to controls using analysis of variance and Tukey's pairwise comparisons ($p<0.05$).

Statistics

The calculated O.D. 50 for each of the treatment groups was compared to controls ($p<0.05$). A total of 59 serum samples from independently challenged mice were analyzed for both IgG and IgM.

One-way Analysis of Variance 90d IgG

Analysis of Variance

Source	DF	SS	MS	F	P
C4	4	0.1163	0.0291	0.92	0.458
Error	54	1.7020	0.0315		
Total	58	1.8183			

```
                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev   -------+---------+---------+---------
 0.0       12    0.3270    0.1413            (----------*---------)
 0.1       11    0.3008    0.1827         (----------*----------)
 1.0       12    0.2374    0.1499   (----------*---------)
 3.0       12    0.2880    0.1522         (---------*---------)
30.0       12    0.3708    0.2424                 (---------*---------)
                                    -------+---------+---------+---------
Pooled StDev =   0.1775                   0.20      0.30      0.40
```

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.00668
Critical value = 3.99

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.1829 0.2353			
1.0	-0.1149 0.2941	-0.1457 0.2725		
3.0	-0.1655 0.2435	-0.1963 0.2219	-0.2551 0.1539	
30.0	-0.2483 0.1607	-0.2791 0.1391	-0.3379 0.0711	-0.2873 0.1217

Descriptive Statistics 90d IgG

Variable	N	N*	Mean	Median	TrMean	StDev
C6	12	0	0.3270	0.2940	0.3222	0.1413
C7	11	1	0.3008	0.2350	0.2908	0.1827
C8	12	0	0.2374	0.2050	0.2210	0.1499
C9	12	0	0.2880	0.2555	0.2728	0.1522
C10	12	0	0.3708	0.2925	0.3431	0.2424

Variable	SE Mean	Minimum	Maximum	Q1	Q3
C6	0.0408	0.1500	0.5520	0.2002	0.4737
C7	0.0551	0.0470	0.6450	0.1450	0.4330
C8	0.0433	0.0430	0.5960	0.1685	0.3143
C9	0.0439	0.1260	0.6020	0.1563	0.4192
C10	0.0700	0.1480	0.8710	0.1670	0.5853

One-way Analysis of Variance 90d IgM

Analysis of Variance

Source	DF	SS	MS	F	P
treatmen	4	0.02701	0.00675	3.13	0.022
Error	54	0.11640	0.00216		
Total	58	0.14341			

```
                                          Individual 95% CIs For Mean
                                          Based on Pooled StDev
Level       N      Mean     StDev   --------+---------+---------+--------
 0.0       12   0.20442   0.05200                    (--------*--------)
 0.1       11   0.16827   0.04506         (--------*--------)
 1.0       12   0.15342   0.02899   (--------*--------)
 3.0       12   0.16608   0.02613        (--------*--------)
30.0       12   0.20508   0.06714                    (--------*--------)
                                    --------+---------+---------+--------
Pooled StDev =  0.04643                   0.150     0.180     0.210
```

Tukey's pairwise comparisons

Family error rate = 0.0500

Individual error rate = 0.00668

Critical value = 3.99

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.01853 0.09082			
1.0	-0.00248 0.10448	-0.03982 0.06953		
3.0	-0.01514 0.09181	-0.05249 0.05687	-0.06614 0.04081	
30.0	-0.05414 0.05281	-0.09149 0.01787	-0.10514 0.00181	-0.09248 0.01448

Descriptive Statistics 90d IgM

Variable	N	N*	Mean	Median	TrMean	StDev
c	12	0	0.2044	0.2080	0.2014	0.0520
0.1	11	1	0.1683	0.1560	0.1649	0.0451
1	12	0	0.15342	0.15050	0.15030	0.02899
3	12	0	0.16608	0.16350	0.16580	0.02613
30	12	0	0.2051	0.2085	0.2083	0.0671

Variable	SE Mean	Minimum	Maximum	Q1	Q3
c	0.0150	0.1400	0.2990	0.1535	0.2415
0.1	0.0136	0.1020	0.2650	0.1450	0.1880
1	0.00837	0.12400	0.21400	0.12625	0.17100
3	0.00754	0.11700	0.21800	0.14825	0.18075
30	0.0194	0.0890	0.2890	0.1410	0.2682

February 1, 1999 EPA Assessment Submission

Attachment #7
Interim Thyroid Histopathology in Mice
(Control and High Dose) from
Keil et al. (1998) Immunotoxicity Studies

A. **Warren 1/13/99 Data Submission**
B. **EPA analysis (Jarabek, 1999)**

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
SUSAN PORTERFIELD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 1, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Review of Interim Pathology Report in Mice from 90-day Immunotoxicity Studies

FROM: Annie M. Jarabek
National Center for Environmental Assessment
RTP (MD-52)

TO: EPA Perchlorate Health Assessment Team

I have reviewed the interim histopathology report received on January 13, 1999 for the mice from the immunotoxicity studies ongoing at the Medical Center of South Carolina (Warren, 1999). These are reported for two 90-day experiments ("A" and "D") and only for the control and high-dose groups. Thus, this analysis is preliminary and limited but nevertheless worthwhile to include at this time since it may add some perspective on interspecies sensitivity. The report is attached.

Three histologic sections (A,B,C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of the thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used. The study pathologist did not read the slides blind, but rather as he notes in the E-mail attached to the report, read the control and high dose specimens to detect a putative morphologic alteration and to characterize the full range of the alterations. Although I understand these points, no mention of a second pathologist to provide QA (per typical NTP SOP) on the study was mentioned. I expect the issue that we have already raised regarding the lack of QA or blind assessment will be resolved in the disposition of the decision regarding a potential pathology working group (PWG) of all the thyroid histopathology, so that I will not belabor the point herein.

In both 90-day experiments ("A" and "D"), the incidence of lesions induced by treatment were 0 in the control and 100% in the 30 mg/kg-day group. Lesions consistent with our proposed mode-of-action were observed, including: colloid depletion, congestion, hypertrophy. Mean values for these lesions are given but the severity range was not provided. The majority of follicles tended to be smaller (a few exceptions on the periphery) with less colloid. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2.0.

These lesions in mice are consistent with those seen in the other species tested and with the proposed mode-of-action for the assessment model. Quantitative interspecies comparison is precluded at this time due to the lack of completed histopathology at the other doses. The Caldwell et al. (1995) study in rats is the only one that tested as high as approximately 22 mg/kg-day, but the difference in severity ratings and lack of statistics for both reports prevents further analysis. In the rabbit developmental study, histopathology was observed at the 30 mg/kg-day dose and this was not the lowest observed effect level. The best data for comparison may be the pending histopathology in the adults of the 2-generation reproductive study in rats, since there was a 30 mg/kg-day testing dose.

In conclusion, this preliminary analysis suggests that the mode-of-action is similar in mice, rabbits and rats. Quantitative interspecies comparison awaits dose-response data in the mice (i.e., histopathology for the remaining dose groups) and possibly a systematic pathology working group (PWG) evaluation of all the histopathology data once they are available.

Attachment

TERRA, Inc.
Toxicology, Ecology, Research, and Risk Assessment

January 13, 1999

Annie Jarabek
NCEA National Center for Environmental Assessment
3210 Highway 54 Catawba Bldg.
RTP Durham, NC 27709-

RE:
Our Case File: MUSC-6872

Dear Ms. Jarabek:

Dave Mattie asked that I send you a copy of the interim pathology report prepared in relation to the perchlorate research effort ongoing at the Medical University of South Carolina. Although my involvement in the research project has been minimal since submitting the grant proposal, as a consultant I have had to stay informed on the issue. I congratulate you and your colleagues for your success in tackling a complex subject in such a systematic and expeditious fashion. I will forward the pathology analysis of the remaining dose groups to you in the near future. Please feel free to call me with your questions or concerns.

Best Regards,

Alan Warren
TERRA, INC.

Mahan Station • 1709 Mahan Drive, Suite E • Tallahassee, Florida 32308 • Phone (850) 309-1330 • Fax (850) 309-1336

John R. Latendresse, D.V.M., Ph.D.
Diplomate of the American College of Veterinary Pathologists

Phone 870-543-7404
E-mail jlatendresse@nctr.fda.gov

Interim Pathology Report
Histopathologic Effects of Ammonium Perchlorate in Thyroid Gland of Mice

Methods

Eight to nine week old male B6C3F1 mice were administered ammonium perchlorate in drinking water for 90 days at 0, 0.1, 1.0, 3.0, and 30 mg/kg/day in two different studies (Studies A and D). For inclusion in this report, only the control and high dose groups from each study were examined. Three histologic sections (A, B, and C) from different levels of the thyroid gland were prepared and submitted for potential histopathologic assessment. Initially, all sections were examined to select the best single section for a detailed evaluation. For consistency in the selection of the region of thyroid gland for the detailed evaluation, only sections of thyroid tissue that contained parathyroid gland were used, when possible. If parathyroid gland was not present, the specimen with the largest area of thyroid gland was used.

Results and Discussion

Morphologies by anatomical site and individual animal are given in the Histopathology Databases (Tables 1 and 2). Thyroid glands from control mice were essentially normal. The follicles were variably sized with complements of relatively large, medium and small colloid-filled lumens. The height of the follicular epithelium was mostly low to medium cuboidal, and the nuclear to cytoplasmic ratio was usually one or less. The cytoplasm of the follicular cells often contained abundant small vacuoles.

The incidence of lesions induced by treatment with ammonium perchlorate is given in the tables 3 and 4. In the 30 mg/kg/day group, although a few peripheral follicles were large with abundant colloid in their lumens, the majority of the follicles tended to be smaller on the average with less colloid compared to controls. Both the inter- and intrafollicular capillaries were mildly congested diffusely, distinguishing them from those of the control thyroid glands. The mildly hypertrophied follicular epithelium was characteristically high cuboidal to low columnar. The nuclear to cytoplasmic ratio of the follicular cells was usually 1.5 to 2. The follicular cells often contained clear perinuclear halos, but the distinct pattern of vacuolization observed in the control group was absent.

Table 3. Study A
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day)	
		0	30
Thyroid follicle	Colloid depletion	0/6 (0)	6/6 (100) [2]*
Capillary	Congestion	0/6 (0)	6/6 (100) [2]
Epithelium, follicular	Hypertrophy	0/6 (0)	6/6 (100) [2]

- [Mean severity]

Table 4. Study D
Incidence (%) of Thyroid Gland Lesions in Mice Exposed to Ammonium Perchlorate

Anatomical Site	Morphology	Dose (mg/kg/day)	
		0	30
Thyroid follicle	Colloid depletion	0/5 (0)	5/5 (100) [2]*
Capillary	Congestion	0/6 (0)	5/5 (100) [1.8]
Epithelium, follicular	Hypertrophy	0/6 (0)	5/5 (100) [1.8]

- [Mean severity]

Inhibition of iodide uptake by the thyroid follicular epithelium has been reported as the mechanism of action of ammonium perchlorate in the rat thyroid gland. Iodination of tyrosine residues of thyroglobulin is one of the essential steps in the production of T3 and T4 (thyroxin). Decreased synthesis of T4 and T3 results in lowered serum concentration that triggers the synthesis and release of TSH from the anterior pituitary gland. TSH receptor activation of cyclic AMP intracellular signaling culminates in hypertrophy of the follicular epithelium. Epithelial hypertrophy, colloid depletion, and the appearance of increased blood flow to the thyroid gland observed in the mice in these studies are consistent with persistent TSH stimulation secondary to deficient production of T3 and/or thyroxin. These observations support a hypothesis of a similar mechanism of action of ammonium perchlorate in the thyroid gland of the mouse that has been shown in the rat.

John R. Latendresse
Diplomate, College of Veterinary Pathologists

Principle investigator: A. Warren, Ph.D.

Table 1
Ammonium Perchlorate
Histopathology Database

Pathologist: J.R. Latendresse, D.V.M., Ph.D.
Diplomate, ACVP

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
A	0	1	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
A	0	2	A	thyroid gland	essentially normal tissue		
A	0	4	A	thyroid gland	essentially normal tissue		
A	0	5	A	thyroid gland	essentially normal tissue		
A	0	6	C	thyroid gland	essentially normal tissue		
A	0	3	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
A	30	28	C	thyroid follicle	colloid depletion	2	
A	30	29	A	thyroid follicle	colloid depletion	2	
A	30	30	A	thyroid follicle	colloid depletion	2	
A	30	25	A	thyroid follicle	colloid depletion	2	
A	30	26	A	thyroid follicle	colloid depletion	2	
A	30	27	A	thyroid follicle	colloid depletion	2	
A	30	25	A	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly dilated and filled with erythrocytes.
A	30	26	A	capillary	congestion	2	
A	30	27	A	capillary	congestion	2	
A	30	28	C	capillary	congestion	2	
A	30	29	A	capillary	congestion	2	
A	30	30	A	capillary	congestion	2	
A	30	25	A	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epiththem is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2.
A	30	26	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	epithelium, follicular	hypertrophy	3	
A	30	28	C	epithelium, follicular	hypertrophy	2	
A	30	29	A	epithelium, follicular	hypertrophy	2	
A	30	30	A	epithelium, follicular	hypertrophy	2	
A	30	27	A	thyroid gland	thyroglossal duct cyst	2	

Principle In[redacted]r: A. Warren, Ph.D.

ı.ıble 2[redacted]
Ammonium Pe[redacted]
Histopathology Da[redacted]ase

Pathologist: J.R. Latendresse, D.V.M., Diplomate, [redacted]

Study ID	Dose mg/kg/day	Animal ID	Slide ID	Site	Diagnosis	Severity	Remarks
D	0	1	A	thyroid adventicia	ectopic thymus		
D	0	3	A	thyroid gland	essentially normal tissue		Follicles are variably sized. Follicular epithelium is low to medium cuboidal with the cytoplasmic to nuclear ratio usually equal to or less than 1. Cytoplasm is often vacuolated.
D	0	4	A	thyroid gland	essentially normal tissue		
D	0	5	A	thyroid gland	essentially normal tissue		
D	0	6	C	thyroid gland	essentially normal tissue		
D	0	2	A	thyroid gland	thyroglossal duct cyst	2	Incidental congenital cyst commonly formed postnatally due to accumulation of proteinaceous fluid in thyroglossal duct remnant.
D	30	25	C	thyroid follicle	colloid depletion	2	Follicles are predominantly small to medium with decreased luminal size and colloid.
D	30	26	A	thyroid follicle	colloid depletion	2	
D	30	27	A	thyroid follicle	colloid depletion	2	
D	30	28	A	thyroid follicle	colloid depletion	2	
D	30	29	C	thyroid follicle	colloid depletion	2	
D	30	25	C	capillary	congestion	2	Inter- and intrafollicular capillaries are prominantly, diffusely dilated and filled with erythrocytes.
D	30	26	A	capillary	congestion	2	
D	30	27	A	capillary	congestion	2	
D	30	28	A	capillary	congestion	2	
D	30	29	C	capillary	congestion	1	
D	30	25	C	epithelium, follicular	hypertrophy	2	Follicles are variably sized. Height of the follicular epiththem is usually high cuboidal to low columnar. Area of follicular cytoplasm is usually 1.5 to 2x greater than controls making cytoplamic to nuclear ratio about 1.5 to 2. Perinuclear halo often present.
D	30	26	A	epithelium, follicular	hypertrophy	2	
D	30	27	A	epithelium, follicular	hypertrophy	2	
D	30	28	A	epithelium, follicular	hypertrophy	2	
D	30	29	C	epithelium, follicular	hypertrophy	1	
D	30	30	C		NOT EXAMINED		RECUT, NOT ENOUGH TISSUE TO EVALUATE.

From: Latendresse, John <JLatendresse@nctr.fda.gov>
To: 'Alan Warren' <awarren@terra1.com>
Date: Monday, January 11, 1999 3:33 PM
Subject: RE: slides

Alan,
I didn't read your message until after I had sent the report out. With few exceptions, I have never been a strong advocate of "blind" histopathology assessment of toxicology studies. Blind reading generally takes much longer, and it can significantly hinder the identification and characterization of lesions induced by exposure to a xenobiotic agent, particularly when they are suttle. With such a study like ammonium perchlorate, I believe that one would get a much more accurate and confident characterization of morphologic alterations by first comparing the high dose and control specimens to establish thresholds for severity scores, for example. Particularly when lesions are suttle, this is an absolutely essential step precluding one's attempt to determine a dose response. To summarize, frankly, in most instances I believe you don't need a blind reading to get a quality, unbiased assessment by the majority of pathologists who characterize morphologic alterations for a living. Often such requests come from scientists who don't understand the process of morphologic assessment. Most pathologists worth their salt actually do some sort of a blind reading anyway, if the study implies a need. For example, after I have carefully compared the morphology of control and high dose specimens, and detect a putative morphologic alteration believed to be due to exposure to a toxicant, I will confirm my observation by examing a pool of unknown specimens. If I can separate the treatment and control specimens based on the morphologic criteria developed during the high dose and control comparison, I proceed with a similar series of exercises in an effort to define a dose response.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 8, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

Dr. Susan Goldhaber
Scientific Coordinator for Perchlorate Peer Review
Research Triangle Institute
3040 Cornwallis Road
P.O. Box 12194
Research Triangle Park, NC 27711

Dear Dr. Goldhaber:

Per our February 1, 1999 submission to Dr. Klaassen via your contract, enclosed please find the second set of analyses we promised (see Table 1). This is another set of new analyses based on data that were not provided in sufficient time to include in the December 31, 1998 external review draft of the document *Perchlorate Environmental Contamination: Toxicology Review and Risk Characterization Based on Emerging Information*. These data represent important information that is being made available as part of completing the original set of studies in the testing strategy. As for the previous data submitted, we will present brief summaries of these data at the peer review meeting. All of these fall into the latter two categories that we described (Category 2 or Category 3) as follows.

1. *Completed EPA analysis:* EPA has finalized its analyses utilizing final audited data from a particular study.

2. *Preliminary EPA analysis:* EPA has either analyzed audited data for individual parameters but the final report audit is not completed, or the analyses EPA performed may not be complete.

3. *Pending data:* These are studies that are in the pipeline. Due dates and thoughts on how these data inform the current effort will be presented.

I will be staying at the contract hotel for the meeting. Should there be any questions on this submission, do not hesitate to contact me there. The NCEA risk assessment team is looking forward to a stimulating and valuable peer review of these data and their anticipated interpretation/integration into the assessment effort. We will be seeing you shortly at the review.

Sincerely,

Annie M. Jarabek
EPA Perchlorate Assessment Team Leader and
Interagency Perchlorate Steering Committee
(IPSC) Executive Committee (NCEA)

Enclosures

cc: **w/o enclosures**

W. Farland, NCEA
Lt. Col. Dan Rogers, IPSC Executive Committee (USAF)
Peter Grevatt, IPSC Executive Committee (OSWER)
Kevin Mayer, IPSC Executive Committee (Region 9)
Mike Osinski, IPSC Executive Committee (OW)

Table 1. Data Analyses in February 8, 1999 Package

Data description	Status of EPA Analysis	Attention Panel Member(s)
1. Different BMD analysis for neurodevelopmental study as suggested by Joe Haseman.	Preliminary — Reanalysis of BMD for neurodevelopmental study (Geller, 1999b) using data as litter-by-litter rather than only those for which hormone and histopathology were performed (Table 6B-7 of document).	Joe Haseman Rochelle Tyl
2. Occupational cross-sectional study of workers exposed via inhalation and an epidemiological study	Preliminary — Manuscripts submitted as accepted on 1/22/99. EPA analysis not complete.	Susan Porterfield Tom Zoeller Charles Emerson
3. Body weight and organ weight audited data and unaudited histopathology from F1 generation in 2-generation reproductive study (Argus, 1998b)	Preliminary — Body weight and organ weight data audited, histopathology is unaudited and final report has not been audited or released. No EPA statistical analysis performed.	Rochelle Tyl Susan Porterfield Tom Zoeller
4. Sheep red blood cell (SRBC) from 14-day experiment (repeat) in immunotoxicity studies	Preliminary — Data audited but final report not released.	Kimber White
5. Correlations between percent of iodide uptake inhibition and hormone perturbations using single dose and repeated 14-day dose PK studies	Preliminary — Data are part of PBPK model development for interspecies extrapolation and completion of mode-of-action motivated model	Mel Andersen

February 8, 1999 EPA Assessment Submission

Attachment #1
Calculations of Quantal Benchmark Doses on Data from Neurodevelopmental Toxicity Study (Argus 1998; York, 1998c) with Full Data

A. EPA Analysis Geller (1999c)

ATTENTION PANEL MEMBER(S):

JOE HASEMAN

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 8 February 1999

Subject: Calculations of Quantal Benchmark Doses on Data from Neurobehavioral Developmental Toxicity Study (Argus, 1998a; York, 1998c) with full data set.

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Memo contains benchmark dose calculations on standard histopathology data set, using all data. Data are considered litter-by-litter; where there was more than one pup considered from a particular litter, the mean severity rating was used, yielding an n = 46 litters. Tables and calculations are for comparison with benchmark calculations for this study initially presented in Table 6B-7 of Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization Based on Emerging Information. Table 6B-7 was calculated with a subset of 36 litters, derived from those animals from whom both histopathology and hormone levels were available.

The inclusion of the additional litters did not significantly affect the benchmark calculations. The benchmark dose and benchmark dose lower confidence limits remained virtually identical to those previously presented. As before, the quantal linear, Weibull, and gamma function models all produced the same fit.

BMDs from Developmental Neurotoxicity Study, PND5, Incidence of Follicular Hyperplasia/Hypertrophy Data. BMDs reflect 10% extra risk with 46 litters.

Function	p of fit, df	BMD	BMDL	LOAEL	BMD: LOAEL	BMDL: LOAEL
Gamma	0.36, 3	0.237	0.101	0.1	2.37	1.01
Logistic	0.36, 3	0.306	0.298	0.1	3.06	2.98
Probit	0.36, 3	0.339	0.303	0.1	3.39	3.03
Quantal Linear	0.36, 3	0.236	0.101	0.1	2.36	1.01
Quantal Quadratic	0.33, 3	0.908	0.528	0.1	9.08	5.28
Weibull	0.36, 3	0.236	0.101	0.1	2.36	1.01

. Gamma Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:32:39 1999

BMDS MODEL RUN

The form of the probability function is:

P[response]=
background+(1-background)*CumGamma[slope*dose,power],
where CumGamma(.) is the cummulative Gamma distribution function

Dependent variable = fh
Independent variable = dose
Power parameter is restricted as power>=1

Total number of observations = 5
Total number of records with missing values = 0

Maximum number of iterations = 250
Relative Function Convergence = 2.22045e-016
Parameter Convergence = 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.482731
Power = 1.2

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.581016	0.121886
Slope	0.444908	0.997695
Power	1	1.90119

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	0.3616	0.5064
Slope	0.3616	1	0.9572
Power	0.5064	0.9572	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.109	3.37943	3	0.184572
Reduced model	-25.3035	8.38885	2	0.015079

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5810	4.648	3	8
0.1000	0.5992	5.992	8	10
1.0000	0.7315	7.315	7	10
3.0000	0.8897	7.118	7	8
10.0000	0.9951	9.951	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3632

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.236814

BMDL = 0.1013

Logistic Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:33:19 1999

BMDS MODEL RUN

The form of the probability function is:

P[response] = 1/[1+EXP(-intercept-slope*dose)]

Dependent variable = fh
Independent variable = dose
Slope parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
intercept = 0.469692
slope = 0.419527

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	0.365989	0.494797
slope	0.56306	0.232602

Asymptotic Correlation Matrix of Parameter Estimates

	intercept	slope
intercept	1	-0.3873
slope	-0.3873	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.0899	3.34115	3	0.341957
Reduced model	-25.3035	8.42713	1	0.0036966

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5905	4.724	3	8
0.1000	0.6040	6.040	8	10
1.0000	0.7169	7.169	7	10
3.0000	0.8865	7.092	7	8
10.0000	0.9975	9.975	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3631

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.306206

BMDL = 0.2978

Multistage Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:33:41 1999

BMDS MODEL RUN

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = fh
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Probit Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:34:00 1999

BMDS MODEL RUN

The form of the probability function is:

P[response] = CumNorm(Intercept+Slope*Dose),

where CumNorm(.) is the cumulative normal distribution function

Dependent variable = fh
Independent variable = dose
Slope parameter is not restricted

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Intercept = 0.334036
Slope = 0.227262

Parameter Estimates

Variable	Estimate	Std. Err.
Intercept	0.242784	0.341629
Slope	0.312349	0.218901

Asymptotic Correlation Matrix of Parameter Estimates

	Intercept	Slope
Intercept	1	-0.6042
Slope	-0.6042	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.0682	3.29771	3	0.347962
Reduced model	-25.3035	8.47057	1	0.0036094

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5959	4.767	3	8
0.1000	0.6080	6.080	8	10
1.0000	0.7106	7.106	7	10
3.0000	0.8810	7.048	7	8
10.0000	0.9996	9.996	10	10

Chi-square = 3.18 DF = 3 P-value = 0.3646

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.338921

BMDL = 0.3028

Quantal Linear Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:34:48 1999

BMDS MODEL RUN

The form of the probability function is:

P[response] = background +
(1-background)*[1-EXP(-slope*dose)]

Dependent variable = fh
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.706952

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.581016	0.143536
Slope	0.444908	0.290282

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope
Background	1	-0.4765
Slope	-0.4765	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.109	3.37943	3	0.33674
Reduced model	-25.3035	8.38885	1	0.0037753

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5810	4.648	3	8
0.1000	0.5992	5.992	8	10
1.0000	0.7315	7.315	7	10
3.0000	0.8897	7.118	7	8
10.0000	0.9951	9.951	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3632

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.236814

BMDL = 0.1013

Quantal Quadratic Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:35:03 1999

BMDS MODEL RUN

The form of the probability function is:

P[response] = background +
(1-background)*[1-EXP(-slope*dose^2)]

Dependent variable = fh
Independent variable = dose

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.0906102

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.624661	0.123807
Slope	0.127549	0.122104

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope
Background	1	-0.4048
Slope	-0.4048	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.1773	3.51586	3	0.318711
Reduced model	-25.3035	8.25242	1	0.0040698

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.6247	4.997	3	8
0.1000	0.6251	6.251	8	10
1.0000	0.6696	6.696	7	10
3.0000	0.8809	7.047	7	8
10.0000	1.0000	10.000	10	10

Chi-square = 3.48 DF = 3 P-value = 0.3239

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.908867

BMDL = 0.5280

Weibull Model, Version Number: 1.1.0b
Input Data File: C:\BMDS\PND5SHALL.(D)

Thu Feb 04 17:35:19 1999

BMDS MODEL RUN

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

Dependent variable = fh
Independent variable = dose
Power parameter is restricted as power>=1

Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 2.22045e-016
Parameter Convergence has been set to: 1.49012e-008

Default Initial Parameter Values
Background = 0.375
Slope = 0.482731
Power = 1.2

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.581016	0.236161
Slope	0.444908	1.17357
Power	1	1.72216

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Slope	Power
Background	1	-0.8411	0.7941
Slope	-0.8411	1	-0.9689
Power	0.7941	-0.9689	1

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	DF	P-value
Full model	-19.4193			
Fitted model	-21.109	3.37943	3	0.184572
Reduced model	-25.3035	8.38885	2	0.015079

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size
0.0000	0.5810	4.648	3	8
0.1000	0.5992	5.992	8	10
1.0000	0.7315	7.315	7	10
3.0000	0.8897	7.118	7	8
10.0000	0.9951	9.951	10	10

Chi-square = 3.19 DF = 3 P-value = 0.3632

Benchmark Dose Computation

Benchmark response = 0.100000

Risk Type = Extra risk

Confidence level = 0.950000

BMD = 0.236814

BMDL = 0.1013

February 8, 1999 EPA Assessment Submission

Attachment #3
Review of Unadudited Terminal Body Weights, Organ Weights, and Ratios; T3, T4, TSH data; and Histopathology in the F1 Generation Rats of the 2-Generation Reproductive Study

A. Body weights, Organ weights, Ratios (York ,1999a)
B. Histopathology and Hormone Data (York, 1999a)
C. Preliminary EPA analysis (Jarabek and Clegg, 1999)

ATTENTION PANEL MEMBER(S):

TOM ZOELLER
ROCHELLE TYL
SUSAN PORTERFIELD
JOE HASEMAN

PRIMEDICA

Argus Research Laboratories, Inc.
905 Sheehy Drive, Building A
Horsham, PA 19044
Telephone: (215) 443-8710
Telefax: (215) 443-8587

February 1, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached is a copy of the summary tables with the F1 generation male and female rats terminal body weights and absolute organ weights and ratios of these organ weights to terminal body weights and to brain weights you requested. Please remember these tables are unaudited and could still change based on the final audit of the study.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Valerie A Sharper

for Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D12 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION MALE PUPS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
TERMINAL BODY WEIGHT	MEAN±S.D.	617.2 ± 76.1	664.6 ± 58.2*	652.6 ± 74.4	638.0 ± 48.8
EPIDIDYMIS LEFT	MEAN±S.D.	0.7617 ±0.0676 [29]b	0.7900 ±0.0695	0.7939 ±0.0651 [29]b	0.7975 ±0.0522
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.3535 ±0.0370 [29]b	0.3702 ±0.0390	0.3654 ±0.0459 [29]b	0.3774 ±0.0403
TESTIS LEFT	MEAN±S.D.	1.8489 ±0.1672 [29]b	1.9460 ±0.1718*	1.9329 ±0.1653 [29]b	1.9670 ±0.1676*
LEFT TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	1.6764 ±0.1636 [29]b	1.7619 ±0.1632	1.7683 ±0.1604 [29]b	1.7813 ±0.1781
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	1.7615 ±0.2457	1.8277 ±0.3127 [28]b	1.7521 ±0.3505	1.7811 ±0.3458
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.7135 ±0.1138	0.7485 ±0.1260	0.7540 ±0.1335	0.7724 ±0.1139
EPIDIDYMIS RIGHT	MEAN±S.D.	0.7669 ±0.0466 [29]b	0.8033 ±0.0596*	0.8070 ±0.0643* [29]b	0.7947 ±0.0487
TESTIS RIGHT	MEAN±S.D.	1.8548 ±0.1572 [29]b	1.9637 ±0.1673	1.9418 ±0.1636 [29]b	1.9518 ±0.1784
PROSTATE	MEAN±S.D.	1.1603 ±0.1966	1.1375 ±0.2382	1.1584 ±0.2321 [29]b	1.2163 ±0.2826
PITUITARY	MEAN±S.D.	0.014 ± 0.002	0.016 ± 0.003**	0.015 ± 0.003	0.016 ± 0.002*
BRAIN	MEAN±S.D.	2.38 ± 0.10	2.44 ± 0.15	2.46 ± 0.11*	2.39 ± 0.10

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
RESEARCH TRIANGLE PARK, NC 27711

February 8, 1999

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Review of Unaudited Terminal Body Weights, Organ Weights, and Ratios; T3, T4, TSH data, and Histopathology in the F1 Generation Rats of the 2-Generation Reproductive Study

FROM: Annie M. Jarabek
NCEA-RTP (MD-52)

Eric Clegg
NCEA-WA (8623)

TO: Perchlorate Risk Assessment Team

We have reviewed the February 1, 1999 submissions (York, 1999b,c) from Primedica / Argus Research Laboratories. York (1999b) provided unaudited terminal body weights, absolute organ weights, and ratios of these organ weights to terminal body weights and to brain weights. York (1999c) provided the hormone and histopathology data. These data could still change based on the final report audit.

Thyroid weights, thyroid to body weight ratio, and thyroid to brain weight were statistically significantly increased in F1 male pups at 3.0 and 30.0 mg/kg-day and in females at 0.3, 3.0 and 30 mg/kg-day. There was also an effect on the pituitary weight and pituitary to brain weight ratio that was significant at 0.3 and 30.0, but not at 3.0 mg/kg-day, although these dose group values were increased over those of controls.

The only treatment-related effect observed was primarily hypertrophy and hyperplasia of the thyroid follicular epithelium. In thyroids with moderate or marked hyperplasia/hypertrophy in the P1 rats, there was a decrease or complete absence of visible colloid. The P1 rats were affected at all dose groups, with incidence and severity increasing in a dose-related manner. In the F1 pups, these changes were noted at the mid and high dose groups. The statistical analyses were run separately for the males versus females and EPA would like to look at the combined data.

There was no apparent dose trend in any of the hormone measures (T3, T4, TSH) as provided, but again EPA would like to run additional alternative analyses using the combined data.

These unaudited data suggest at this time a corroboration of the thyroid effects seen in pups of the neurodevelopmental study. More rigorous analysis to look at the severity scoring and alternative analyses of the combined data are recommended. The incidence of histopathology suggests support of an effect at 0.3 mg/kg-day seen in the organ weight data. The hormone data may not sufficiently analyzed at this time but do not appear to correspond with the histopathology and weight data. The results of the inter-laboratory validation study for the hormone analyses may be informative to interpreting this discrepancy.

Attachments

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D12 (PAGE 2): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION MALE PUPS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
LIVER	MEAN±S.D.	24.72 ± 4.03	27.30 ± 3.54**	26.18 ± 3.26	25.28 ± 3.17 [26]b
KIDNEY PAIRED	MEAN±S.D.	4.84 ± 0.59	5.23 ± 0.45*	5.18 ± 0.64*	5.11 ± 0.49
ADRENAL PAIRED	MEAN±S.D.	0.069 ± 0.011	0.071 ± 0.011	0.073 ± 0.013	0.071 ± 0.010
SPLEEN	MEAN±S.D.	1.01 ± 0.16	1.08 ± 0.22	1.12 ± 0.18*	1.08 ± 0.12*
THYROID	MEAN±S.D.	0.036 ± 0.005	0.041 ± 0.009	0.044 ± 0.005**	0.063 ± 0.012** [26]c
HEART	MEAN±S.D.	1.91 ± 0.21	2.01 ± 0.27	2.04 ± 0.22	1.96 ± 0.19

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead.
b. Excludes value for rat 7095, value was not recorded.
c. Excludes values for rats that had organs damaged (weight affected).
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D13 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
TERMINAL BODY WEIGHT	MEAN+S.D.	617.2 + 76.1	664.6 + 58.2*	652.6 + 74.4	638.0 + 48.8
EPIDIDYMIS LEFT	MEAN+S.D.	0.124 + 0.014 [29]b	0.118 + 0.017	0.122 + 0.017 [29]b	0.125 + 0.010
CAUDA EPIDIDYMIS LEFT	MEAN+S.D.	0.057 + 0.008 [29]b	0.056 + 0.009	0.057 + 0.009 [29]b	0.059 + 0.007
TESTIS LEFT	MEAN+S.D.	0.302 + 0.033 [29]b	0.294 + 0.038	0.298 + 0.039 [29]b	0.310 + 0.028
LEFT TESTIS MINUS TUNICA ALBUGINEA	MEAN+S.D.	0.273 + 0.032 [29]b	0.265 + 0.034	0.272 + 0.038 [29]b	0.280 + 0.028
SEMINAL VESICLES WITH FLUID	MEAN+S.D.	0.288 + 0.052	0.275 + 0.058 [28]b	0.273 + 0.069	0.281 + 0.056
SEMINAL VESICLES WITHOUT FLUID	MEAN+S.D.	0.116 + 0.025	0.112 + 0.021	0.117 + 0.025	0.121 + 0.020
EPIDIDYMIS RIGHT	MEAN+S.D.	0.126 + 0.016 [29]b	0.121 + 0.016	0.123 + 0.017 [29]b	0.125 + 0.011
TESTIS RIGHT	MEAN+S.D.	0.303 + 0.037 [29]b	0.297 + 0.038	0.300 + 0.038 [29]b	0.307 + 0.032
PROSTATE	MEAN+S.D.	0.189 + 0.032	0.171 + 0.033	0.179 + 0.042 [29]b	0.190 + 0.040
PITUITARY c	MEAN+S.D.	2.267 + 0.336	2.437 + 0.394	2.353 + 0.517	2.470 + 0.368
BRAIN	MEAN+S.D.	0.390 + 0.048	0.369 + 0.030	0.379 + 0.037	0.376 + 0.028

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).

c. Value was multiplied by 1000.

* Significantly different from the carrier group value ($p \leq 0.05$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D13 (PAGE 2): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP		1	2	3	4
TARGET DOSAGE (MG/KG/DAY)		0 (CARRIER)	0.3	3.0	30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
LIVER	MEAN±S.D.	3.996 ± 0.330	4.113 ± 0.436	4.016 ± 0.268	3.951 ± 0.368 [26]b
KIDNEY PAIRED	MEAN±S.D.	0.786 ± 0.065	0.789 ± 0.075	0.795 ± 0.066	0.800 ± 0.056
ADRENAL PAIRED	MEAN±S.D.	0.010 ± 0.000	0.010 ± 0.000	0.011 ± 0.002	0.010 ± 0.002
SPLEEN	MEAN±S.D.	0.165 ± 0.021	0.163 ± 0.033	0.172 ± 0.024	0.170 ± 0.017
THYROID c	MEAN±S.D.	5.935 ± 0.840	6.116 ± 1.092	6.705 ± 0.600**	9.888 ± 1.823** [26]d
HEART	MEAN±S.D.	0.311 ± 0.036	0.303 ± 0.037	0.316 ± 0.035	0.306 ± 0.025

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes value for rat 7095, value was not recorded.

c. Value was multiplied by 1000.

d. Excludes values for rats that had organs damaged (weight affected).

** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D14 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO BRAIN WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
BRAIN WEIGHT	MEAN±S.D.	2.38 ± 0.10	2.44 ± 0.15	2.46 ± 0.11*	2.39 ± 0.10
EPIDIDYMIS LEFT	MEAN±S.D.	32.02 ± 2.72 [29]b	32.50 ± 3.23	32.30 ± 3.01 [29]b	33.45 ± 2.41
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	14.86 ± 1.53 [29]b	15.22 ± 1.70	14.86 ± 1.93 [29]b	15.82 ± 1.62
TESTIS LEFT	MEAN±S.D.	77.68 ± 6.11 [29]b	79.95 ± 6.80	78.62 ± 7.18 [29]b	82.44 ± 6.76
LEFT TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	70.43 ± 6.09 [29]b	72.40 ± 6.71	71.93 ± 6.92 [29]b	74.63 ± 6.89
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	74.09 ± 10.98	75.29 ± 13.09 [28]b	71.44 ± 15.18	74.45 ± 13.08
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	30.01 ± 4.97	30.71 ± 4.76	30.75 ± 5.94	32.33 ± 4.46
EPIDIDYMIS RIGHT	MEAN±S.D.	32.25 ± 1.92 [29]b	33.06 ± 2.95	32.85 ± 3.06 [29]b	33.33 ± 2.28
TESTIS RIGHT	MEAN±S.D.	77.93 ± 5.61 [29]b	80.68 ± 6.67	78.96 ± 6.92 [29]b	81.80 ± 7.17
PROSTATE	MEAN±S.D.	48.72 ± 8.11	46.78 ± 10.00	47.08 ± 9.43 [29]b	50.89 ± 11.26
PITUITARY	MEAN±S.D.	0.58 ± 0.10	0.66 ± 0.12*	0.62 ± 0.12	0.66 ± 0.09*
LIVER	MEAN±S.D.	1037.23 ±158.37	1120.77 ±135.98	1063.74 ±116.27	1062.09 ±122.13 [26]c

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/BRAIN WEIGHT) x 100.

[ ] = NUMBER OF VALUES AVERAGED

a. Excludes values for rats that were found dead or moribund sacrificed.

b. Excludes values for rats that had abnormal organs (weight affected) or organs damaged (weight affected).

c. Excludes value for rat 7095, value was not recorded.

* Significantly different from the carrier group value ($p \leq 0.05$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE D14 (PAGE 2): RATIOS (%) OF BRAIN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION MALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
INCLUDED IN ANALYSES	N	30	30	30	27a
KIDNEY PAIRED	MEAN±S.D.	203.09 ± 22.84	214.72 ± 17.71	210.10 ± 21.08	214.30 ± 19.96
ADRENAL PAIRED	MEAN±S.D.	2.91 ± 0.46	2.93 ± 0.44	2.98 ± 0.54	3.00 ± 0.51
SPLEEN	MEAN±S.D.	42.56 ± 6.02	44.51 ± 9.10	45.78 ± 7.49	45.40 ± 4.73
THYROID	MEAN±S.D.	1.52 ± 0.21	1.68 ± 0.37	1.77 ± 0.17**	2.64 ± 0.52** [26]b
HEART	MEAN±S.D.	80.26 ± 9.44	82.59 ± 11.57	83.18 ± 8.57	81.86 ± 6.64

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/BRAIN WEIGHT) x 100.
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for rats that were found dead or moribund sacrificed.
b. Excludes values for rats that had organs damaged (weight affected).
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E27 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
PREGNANT	N	21	27	28	27
INCLUDED IN ANALYSES	N	20a	27b	28	25c,d
TERMINAL BODY WEIGHT	MEAN±S.D.	354.0 ± 18.6	361.0 ± 22.6	368.0 ± 21.4	367.2 ± 21.0
PITUITARY	MEAN±S.D.	0.016 ± 0.003	0.016 ± 0.003	0.016 ± 0.004	0.016 ± 0.004
BRAIN	MEAN±S.D.	2.20 ± 0.11	2.23 ± 0.10	2.23 ± 0.08	2.19 ± 0.11
LIVER	MEAN±S.D.	19.92 ± 2.10	20.27 ± 1.73	19.67 ± 1.39	19.11 ± 1.76
KIDNEY PAIRED	MEAN±S.D.	3.31 ± 0.27	3.32 ± 0.26	3.38 ± 0.25	3.26 ± 0.32
ADRENAL PAIRED	MEAN±S.D.	0.112 ± 0.013	0.107 ± 0.013	0.112 ± 0.011	0.103 ± 0.014*
SPLEEN	MEAN±S.D.	0.74 ± 0.12	0.76 ± 0.12	0.77 ± 0.10	0.77 ± 0.09
OVARY PAIRED	MEAN±S.D.	0.126 ± 0.027	0.126 ± 0.027	0.130 ± 0.029	0.131 ± 0.029
THYROID	MEAN±S.D.	0.022 ± 0.003	0.025 ± 0.004*	0.028 ± 0.004** [27]	0.033 ± 0.005**
HEART	MEAN±S.D.	1.37 ± 0.13	1.38 ± 0.10	1.39 ± 0.12	1.39 ± 0.12

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for dam 7212, which did not deliver a litter; only one early resorption was present <u>in utero</u> on day 25 of gestation.

b. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning.
c. Excludes values for dam 7293, which did not deliver a litter; only three early resorptions was present <u>in utero</u> on day 25 of gestation.
d. Excludes values for dam 7295, which was found dead on day 22 of gestation.
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E28 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
PREGNANT	N	21	27	28	27
INCLUDED IN ANALYSES	N	20a	27b	28	25c,d
TERMINAL BODY WEIGHT	MEAN±S.D.	354.0 ± 18.6	361.0 ± 22.6	368.0 ± 21.4	367.2 ± 21.0
PITUITARY e	MEAN±S.D.	4.469 ± 0.688	4.559 ± 0.752	4.315 ± 1.098	4.462 ± 0.997
BRAIN	MEAN±S.D.	0.622 ± 0.038	0.621 ± 0.048	0.607 ± 0.034	0.599 ± 0.042
LIVER	MEAN±S.D.	5.632 ± 0.575	5.652 ± 0.339	5.350 ± 0.339	5.206 ± 0.385*
KIDNEY PAIRED	MEAN±S.D.	0.934 ± 0.074	0.923 ± 0.073	0.918 ± 0.059	0.887 ± 0.080
ADRENAL PAIRED	MEAN±S.D.	0.030 ± 0.004	0.029 ± 0.005	0.031 ± 0.004	0.028 ± 0.004
SPLEEN	MEAN±S.D.	0.208 ± 0.034	0.211 ± 0.035	0.210 ± 0.029	0.208 ± 0.027
OVARY PAIRED	MEAN±S.D.	0.036 ± 0.008	0.034 ± 0.008	0.035 ± 0.008	0.035 ± 0.008
THYROID e	MEAN±S.D.	6.279 ± 0.917	7.032 ± 1.224*	7.707 ± 1.172** [27]	9.018 ± 1.333**
HEART	MEAN±S.D.	0.388 ± 0.034	0.382 ± 0.029	0.377 ± 0.026	0.377 ± 0.028

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for dam 7212, which did not deliver a litter; only one early resorption was present <u>in utero</u> on day 25 of gestation.

b. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning.
c. Excludes values for dam 7293, which did not deliver a litter; only three early resorptions was present <u>in utero</u> on day 25 of gestation.
d. Excludes values for dam 7295, which was found dead on day 22 of gestation.
e. Value was multiplied by 1000.
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

PROTOCOL 1416-001: ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION) REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS

TABLE E29 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO BRAIN WEIGHT - SUMMARY - F1 GENERATION FEMALE RATS

DOSAGE GROUP TARGET DOSAGE (MG/KG/DAY)		1 0 (CARRIER)	2 0.3	3 3.0	4 30.0
RATS TESTED	N	30	30	30	30
PREGNANT	N	21	27	28	27
INCLUDED IN ANALYSES	N	20a	27b	28	25c,d
BRAIN WEIGHT	MEAN±S.D.	2.20 ± 0.11	2.23 ± 0.10	2.23 ± 0.08	2.19 ± 0.11
PITUITARY	MEAN±S.D.	0.72 ± 0.12	0.73 ± 0.13	0.71 ± 0.18	0.74 ± 0.15
LIVER	MEAN±S.D.	908.83 ±100.57	909.06 ± 86.73	883.81 ± 65.55	873.83 ± 95.77
KIDNEY PAIRED	MEAN±S.D.	150.81 ± 11.38	148.76 ± 13.01	151.69 ± 10.18	148.88 ± 16.50
ADRENAL PAIRED	MEAN±S.D.	5.12 ± 0.59	4.78 ± 0.59	5.03 ± 0.48	4.70 ± 0.70
SPLEEN	MEAN±S.D.	33.77 ± 6.24	34.00 ± 5.60	34.75 ± 5.04	34.95 ± 4.00
OVARY PAIRED	MEAN±S.D.	5.75 ± 1.23	5.66 ± 1.30	5.84 ± 1.38	6.00 ± 1.35
THYROID	MEAN±S.D.	1.01 ± 0.14	1.13 ± 0.18*	1.28 ± 0.19** [27]	1.51 ± 0.23**
HEART	MEAN±S.D.	62.39 ± 6.23	61.60 ± 3.89	62.24 ± 4.75	63.32 ± 5.47

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Excludes values for dam 7212, which did not deliver a litter; only one early resorption was present in utero on day 25 of gestation.

b. Excludes values for rat 7253, which was moribund sacrificed on day 62 postweaning.
c. Excludes values for dam 7293, which did not deliver a litter; only three early resorptions was present in utero on day 25 of gestation.
d. Excludes values for dam 7295, which was found dead on day 22 of gestation.
e. Value was multiplied by 1000.
* Significantly different from the carrier group value ($p \leq 0.05$).
** Significantly different from the carrier group value ($p \leq 0.01$).

PRIMEDICA

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February 01, 1999

Joan Dollarhide
Toxicology Excellence for Risk Assessment (TERA)
4303 Hamilton Avenue
Cincinnati, Ohio 45223

Telephone: (606) 428-2744
Fax: (606) 428-3386

RE: Protocol 1416-001 - Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats

Dear Joan:

Attached are unaudited copies of summary tables with the P1 and F1 generation male and female rats histopatholgy and Ray Brown's draft report. The hormone data sent to us from AniLytics has also been statistically analyzed by us put into a table for you. I also have enclosed another copy of the organ weigh and organ weight ratios. Please remember these tables are unaudited and could still change based on the final audit of the study.

If you have any questions, please do not hesitate to contact me.

Sincerely,

Ray

Raymond G. York, Ph.D., DABT
Associate Director of Research
and Study Director

RGY:rgy
Enc.

P1 Female-adult

		TSH	T3	T4
Group 1	Mean	2.054	57.77	2.126
	S.D.	0.873	28.204	0.677
Group 2	Mean	2.213	64.789	2.903**
	S.D.	0.987	29.277	1.039
Group 3	Mean	1.99	56.35	2.924**
	S.D.	0.773	13.992	0.841
Group 4	Mean	2.174	60.373	2.421
	S.D.	0.744	21.97	0.792

P1 Male-adult

		TSH	T3	T4
Group 1	Mean	1.53	72.546	4.641
	S.D.	0.957	11.228	0.583
Group 2	Mean	1.353	87.389**	4.726
	S.D.	0.638	16.257	0.817
Group 3	Mean	1.487	88.452**	4.744
	S.D.	0.815	18.634	0.79
Group 4	Mean	3.871**	78.57	3.578**
	S.D.	3.495	14.363	0.86

F1 Female-pup

Group 1	Mean	1.12	105.954	4.27
	S.D.	0.51	13.075	1.019
Group 2	Mean	1.188	109.922	4.865*
	S.D.	0.352	13.066	0.946
Group 3	Mean	1.141	109.293	4.324
	S.D.	0.375	13.568	0.778
Group 4	Mean	1.301	97.581*	3.913
	S.D.	0.351	11.046	0.983

F1 Male-pup

Group 1	Mean	1.237	105.897	4.403
	S.D.	0.448	9.976	1.013
Group 2	Mean	0.941**	111.15	4.615
	S.D.	0.342	16.372	0.979
Group 3	Mean	0.877**	109.81	4.533
	S.D.	0.254	15.693	0.789
Group 4	Mean	1.27	107.398	4.525
	S.D.	0.381	16.06	1.086

1416-001

F1 Female-adult

		TSH	T3	T4
Group 1	Mean	1.625	61.47	2.219
	S.D.	1.006	25.235	1.025
Group 2	Mean	1.223	51.191	2.026
	S.D.	0.663	21.939	0.836
Group 3	Mean	1.647	53.41	2.273
	S.D.	0.882	19.582	1.053
Group 4	Mean	2.12*	56.856	2.131
	S.D.	0.692	18.888	0.863

F1 Male-adult

		TSH	T3	T4
Group 1	Mean	2.507	82.5	3.784
	S.D.	1.01	8.677	0.547
Group 2	Mean	2.164	81.27	4.214*
	S.D.	1.044	15.435	0.862
Group 3	Mean	2.303	83.232	4.204*
	S.D.	1.729	16.496	0.865
Group 4	Mean	5.176**	82.969	2.777**
	S.D.	2.523	13.378	0.716

F2 Female-pup

Group 1	Mean	0.938	108.391	3.402
	S.D.	0.284	21.091	0.724
Group 2	Mean	0.911	107.395	3.336
	S.D.	0.29	13.002	0.787
Group 3	Mean	0.959	107.856	4.151**
	S.D.	0.222	20.657	1.046
Group 4	Mean	0.966	98.846	3.834
	S.D.	0.218	23.988	0.832

F2 Male-pup

Group 1	Mean	0.818	106.346	3.231
	S.D.	0.193	18.27	0.839
Group 2	Mean	0.876	108.035	3.307
	S.D.	0.255	14.633	0.859
Group 3	Mean	0.949	119.532*	3.794
	S.D.	0.277	20.083	0.881
Group 4	Mean	0.959	107.094	3.422
	S.D.	0.214	21.357	0.804

Histopathology - P1 Generation Male Rats

Hyperplasia/hypertrophy, follicular epithelium (N=30):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	0	2	1	0
-mild	1	5	5	0
-moderate	1	2	11	1
-marked	0	0	8	29
Total	2	9	25**	30**

** Significantly different from control $p \leq 0.01$

Histopathology - P1 Generation Female Rats

Hyperplasia/hypertrophy, follicular epithelium (N=22 to 28):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	2	4	4	0
-mild	1	3	8	0
-moderate	1	3	8	2
-marked	0	0	0	22
Total	4	10	20**	24**

** Significantly different from control $p \leq 0.01$

Histopathology - F1 Generation Male Rats

Hyperplasia/hypertrophy, follicular epithelium (N=30):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	0	0	3	0
-mild	3	4	5	0
-moderate	2	4	5	3
-marked	0	0	6	23
Total	5	8	19**	26**

** Significantly different from control $p<0.01$

Histopathology - F1 Generation Female Rats

Hyperplasia/hypertrophy, follicular epithelium (N=20 to 28):

Severity	0 (Carrier)	0.3 mg/kg/day	3.0 mg/kg/day	30.0 mg/kg/day
-minimal	4	5	6	1
-mild	2	1	6	2
-moderate	0	0	1	10
-marked	0	0	0	11
Total	6	6	13	24**

** Significantly different from control $p<0.01$

RESEARCH
PATHOLOGY
SERVICES, INC.

January 25, 1999

TO: Raymond G. York, Ph.D., D.A.B.T.
Argus Research Laboratories, Inc.
905 Sheehy Drive
Horsham, PA 19044

FROM: W. Ray Brown, D.V.M., Ph.D. WRBrown 1-25-99
Veterinary Pathologist

SUBJECT: Oral (Drinking Water) Two-Generation (One Litter per Generation) Reproduction Study of Ammonium Perchlorate in Rats
Protocol 1416-001 - Interim Histopathology Report
Preliminary Histopathology Data - P1 and F1 Generation Adult Rats

Method:

Microscopic examination was made of the specified tissues from parental male and parental female Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats in four treatment groups used in a two-generation reproduction study of ammonium perchlorate. A brief outline of the study design is shown below.

Group	Number of Parental Rats/Group P1	F1	Target Dosage (mg/kg/day)	Concentration (µg/mL)[a]
1	30M,28F	30M,20F	0 (Carrier)[b]	0
2	30M,22F	30M,27F	0.3	2.3
3	30M,26F	30M,28F	3.0	22.8
4	30M,24F	30M,25F	30.0	227.4

[a]Based on average water consumption of 33 mL/animal/day (132 mL/kg/day) for a 250g rat. These concentrations were adjusted weekly based on actual body weight and water consumption levels recorded the previous week. The test article was considered 100% pure for the purpose of dosage calculations.
[b]Reverse osmosis membrane processed deionized water (R. O. deionized water).

P1 generation rats were given continual access to the test substance in the drinking water beginning at least 70 days before cohabitation (approximately six weeks of age) and continuing through the day before sacrifice. Rats of the F1 generation were given the same test substance concentrations as their respective

P1 generation sires and dams once daily beginning at weaning and continued through the day before sacrifice. Rats of this generation may have been exposed *in utero* during gestation and via maternal milk and water during the postpartum period.

Results:

The type and incidence by degree of severity of histomorphologic changes in the P1 and F1 generation parental male and female rats are presented in Tables 1 and 2, respectively.

The only tissue in which a compound-related change occurred was the thyroid gland and the change occurred in male and female rats of the P1 and F1 generations. The treatment-related change in the thyroid was primarily hypertrophy and hyperplasia of the thyroid follicular epithelium. In many of the affected thyroids, there were increased numbers of small follicles (hyperplasia) and these follicles had enlarged (hypertrophied) follicular epithelial cells. In the thyroids with a moderate or marked hyperplasia/hypertrophy, there was a decrease or complete absence of visible colloid in the affected follicles. The incidence by degree of severity of the thyroid changes in the P1 generation male and female rats is presented in the following text table.

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Rats/Group:	30	30	30	30	28	22	26	24
THYROID:								
-hyperplasia/hypertrophy, follicular epithelium								
minimal	0	2	1	0	2	4	4	0
mild	1	5	5	0	1	3	8	0
moderate	1	2	11	1	1	3	8	2
marked	0	0	8	29	0	0	0	22
Total Incidence	2	9	25	30	4	10	20	24

The same information is presented in the following text table for the F1 generation male and female rats.

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Rats/Group:	30	30	30	30	20	27	28	25
THYROID:								
-hyperplasia/hypertrophy, follicular epithelium								
minimal	0	0	3	0	4	5	6	1
mild	3	4	5	0	2	1	6	2
moderate	2	4	5	3	0	0	1	10
marked	0	0	6	23	0	0	0	11
Total Incidence	5	8	19	26	6	6	13	24

The degree of the thyroid change ranged from minimal to marked and generally occurred in a dose-related fashion. For the P1 generation, the incidence and severity of the thyroid hypertrophy and hyperplasia was considered to be increased in all compound-treated groups, while in the F1 generation, the treatment-related effect (increased incidence and severity of thyroid hyperplasia and hypertrophy) was judged to have occurred in the mid and high dosage groups. The incidence in the low-dose F1 generation rats was considered to be comparable to the controls. There were a few control male and female rats in both generations with a similar type of histomorphologic change in the thyroid, but with a lower incidence and severity as compared to the affected compound-treated P1 and F1 generation male and female rats listed above.

There were no other tissues with compound-related changes in any of the P1 or F1 generation parental rats given 30 mg/kg/day of the test article for the respective treatment periods. The other changes that were observed were typical of those that occur spontaneously in male and female rats used in reproductive studies and their type or incidence was not considered to have been influenced by compound administration. These changes are also listed and summarized in the attached Tables 1 and 2.

<u>Summary:</u>

Microscopic examination was made of the specified tissues from parental male and female rats of the P1 and F1 generations of an oral (drinking water) two generation reproduction study of ammonium perchlorate. The dosages used in the study were 0 (carrier), 0.3, 3.0 or 30.0 mg/kg/day.

Treatment-related microscopic changes were observed in the thyroid gland of the parental male and female rats of the P1 generation and F1 generation rats given the test article.

The treatment-related change consisted of an increased incidence and severity of thyroid follicular epithelial hyperplasia and hypertrophy in male and female rats of all compound-treatment groups in the P1 generation and in the mid and high dosage groups of the F1 generation. There was a low incidence of a similar type thyroid change in a few control animals, but the incidence and severity of the above-mentioned groups was considered to have been increased in a dose-related manner and to be compound-related.

All other microscopic changes observed in the other organs and tissues specified for examination from the P1 and F1 generation parental male and female rats given 30 mg/kg/day of ammonium perchlorate were considered to have occurred spontaneously and to be unrelated to treatment.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
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TABLE 1

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
ADRENAL GLANDS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	24	0	0	19	20	0	0	18
-hypertrophy, cortical, focal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[1]
minimal	0	0	0	1	1	0	0	1
-vacuolation, cortical, focal	[6]	[0]	[0]	[11]	[8]	[0]	[0]	[5]
minimal	4	0	0	7	8	0	0	5
mild	2	0	0	4	0	0	0	0
AORTA:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
BONE (FEMUR):								
NO. EXAMINED	29	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	23
-cyst(s), epiphysis	0	0	0	0	0	0	0	1
-fibrosis, epiphysis, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
BONE MARROW (FEMUR):								
NO. EXAMINED	29	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	24
-lymphoma, malignant	0	0	0	1	0	0	0	0
BRAIN:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
CECUM:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	28	0	0	30	24	0	0	23
-edema, submucosa	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
-hyperplasia, lymphoid	[2]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
mild	2	0	0	0	0	0	0	0
-inflammation, chronic, mucosa	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
-parasite(s), lumen	0	0	0	0	2	0	0	1
CERVIX:								
NO. EXAMINED					28	0	0	23
NO. NORMAL					28	0	0	23

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
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TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
COLON:								
NO. EXAMINED	30	0	0	30	28	0	0	23
NO. NORMAL	29	0	0	30	27	0	0	18
-parasite(s), lumen	1	0	0	0	1	0	0	5
DUODENUM:								
NO. EXAMINED	29	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	30	28	0	0	24
EAR(S):								
NO. EXAMINED	0	0	0	0	4	2	3	0
NO. NORMAL	0	0	0	0	1	0	0	0
-chondritis, auricular	[0]	[0]	[0]	[0]	[2]	[2]	[3]	[0]
mild	0	0	0	0	0	0	1	0
moderate	0	0	0	0	2	0	0	0
marked	0	0	0	0	0	2	2	0
-dermatitis, chronic	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
EPIDIDYMIDES:								
NO. EXAMINED	30	1	1	30				
NO. NORMAL	17	0	0	19				
-hypospermia	[0]	[0]	[1]	[0]				
marked	0	0	1	0				
-infiltration, mononuclear-cell, focal	[13]	[0]	[1]	[11]				
minimal	13	0	1	11				
-necrotic germ cells	0	1	0	0				
ESOPHAGUS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
EYES:								
NO. EXAMINED	30	1	1	30	28	0	0	24
NO. NORMAL	30	0	0	28	28	0	0	24
-atrophy, retinal, focal	[0]	[1]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	1	0	2	0	0	0	0
-keratitis, chronic	[0]	[0]	[1]	[0]	[0]	[0]	[0]	[0]
mild	0	0	1	0	0	0	0	0
-mineralization, cornea, focal	[0]	[0]	[1]	[0]	[0]	[0]	[0]	[0]
minimal	0	0	1	0	0	0	0	0
HEART:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	21	0	0	15	28	0	0	24

[ ] = Total incidence of specified lesion, all grades.

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
HEART (Continued):								
-fibrosis, myocardial, focal	[7]	[0]	[0]	[10]	[0]	[0]	[0]	[0]
minimal	7	0	0	8	0	0	0	0
mild	0	0	0	2	0	0	0	0
-hemosiderosis	[4]	[0]	[0]	[6]	[0]	[0]	[0]	[0]
minimal	4	0	0	6	0	0	0	0
-inflammation, chronic, multifocal	[3]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	3	0	0	5	0	0	0	0
ILEUM:								
NO. EXAMINED	30	0	0	28	27	0	0	24
NO. NORMAL	29	0	0	28	27	0	0	24
-Advanced autolysis precludes evaluation	1	0	0	0	0	0	0	0
JEJUNUM:								
NO. EXAMINED	30	0	0	29	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	24
-Advanced autolysis precludes evaluation	1	0	0	0	0	0	0	0
KIDNEYS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	14	0	0	17	23	0	0	16
-cyst(s), medulla	2	0	0	0	0	0	0	0
-degeneration/basophilia, cortical tubules, focal	[7]	[0]	[0]	[7]	[0]	[0]	[0]	[0]
minimal	6	0	0	6	0	0	0	0
mild	1	0	0	1	0	0	0	0
-dilatation, cortical tubules	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	0	0	0	0	0	0	0	1
-dilatation, pelvis	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
mild	0	0	0	1	0	0	0	0
-fibrosis, focal	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	0	0	0	0	0	0	0	1
-glomerulonephritis, chronic	[1]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	1	0	0	3	0	0	0	0
-hyaline droplets, cortical tubules	[2]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
mild	2	0	0	1	0	0	0	0
moderate	0	0	0	1	0	0	0	0
-infiltration, mononuclear-cell, focal/multifocal	[10]	[0]	[0]	[5]	[2]	[0]	[0]	[1]
minimal	10	0	0	5	2	0	0	1

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
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TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 28	2 F 22	3 F 26	4 F 24
KIDNEYS (Continued):								
-mineralization, multifocal	[0]	[0]	[0]	[0]	[2]	[0]	[0]	[6]
minimal	0	0	0	0	2	0	0	6
-mineralization, pelvis	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
-vacuolation, cortical tubules	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
mild	0	0	0	0	0	0	0	1
LIVER:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	18	0	0	19	24	0	0	19
-cellular alteration, clear-cell, focal	[1]	[0]	[0]	[1]	[0]	[0]	[0]	[1]
minimal	1	0	0	1	0	0	0	1
-hematopoiesis, extramedullary	[1]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	2	0	0	0	0
-inflammation, chronic, focal/multifocal	[9]	[0]	[0]	[9]	[4]	[0]	[0]	[3]
minimal	9	0	0	9	4	0	0	3
-necrosis, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[2]
minimal	1	0	0	0	0	0	0	2
-vacuolation, hepatocellular, periportal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
LUNG:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	19	0	0	17	17	0	0	12
-alveolitis, acute, focal/multifocal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-hypertrophy, arterial	[1]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
mild	0	0	0	1	0	0	0	0
moderate	1	0	0	0	0	0	0	0
-infiltration, eosinophilic, perivascular and peribronchial	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[2]
minimal	0	0	0	0	0	0	0	2
-inflammation, interstitial, focal	[1]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	1	0	0	1	1	0	0	0
-macrophages, alveoli, focal	[9]	[0]	[0]	[11]	[9]	[0]	[0]	[11]
minimal	9	0	0	9	8	0	0	10
mild	0	0	0	2	1	0	0	1

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
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TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
LUNG (Continued):								
-proliferation, lymphoid, peribronchial/perivascular	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
LYMPH NODE, MANDIBULAR:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	18	0	0	18	11	0	0	8
-hyperplasia, lymphocytic/plasmacytic	[12]	[0]	[0]	[12]	[17]	[0]	[0]	[16]
minimal	5	0	0	5	5	0	0	5
mild	2	0	0	5	3	0	0	6
moderate	5	0	0	2	9	0	0	5
-lymphadenopathy, cystic	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
LYMPH NODE, MESENTERIC:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	27	0	0	30	23	0	0	22
-congestion/erythrophagocytosis	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
-edema	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
moderate	0	0	0	0	1	0	0	0
-hemorrhage	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
mild	1	0	0	0	0	0	0	0
-histiocytosis	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[2]
mild	0	0	0	0	3	0	0	1
moderate	0	0	0	0	0	0	0	1
-hyperplasia, lymphoid	[1]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	1	0	0	0	1	0	0	0
LYMPHORETICULAR SYSTEM:								
-lymphoma, malignant	0	0	0	1	0	0	0	0
MAMMARY GLAND:								
NO. EXAMINED	19	0	0	22	28	1	0	24
NO. NORMAL	19	0	0	22	26	0	0	23
-adenocarcinoma	0	0	0	0	0	1/2	0	0
-cystic gland/duct	0	0	0	0	1	0	0	1

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
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TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 28	2 F 22	3 F 26	4 F 24
MAMMARY GLAND (Continued):								
-inflammation, subacute	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
MUSCLE, SKELETAL:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	29	0	0	29	28	0	0	24
-inflammation, chronic, focal	[1]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
mild	1	0	0	0	0	0	0	0
NERVE, SCIATIC:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
OVARIES:								
NO. EXAMINED					28	0	0	24
NO. NORMAL					28	0	0	24
PALATE:								
NO. EXAMINED	0	0	0	1	0	0	0	0
NO. NORMAL	0	0	0	0	0	0	0	0
-abscess	0	0	0	1	0	0	0	0
-fracture	0	0	0	1	0	0	0	0
PANCREAS:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	17	0	0	19	28	0	0	24
-atrophy, acinar, focal	[3]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	2	0	0	4	0	0	0	0
moderate	1	0	0	1	0	0	0	0
-fibrosis, islet, focal	[8]	[0]	[0]	[8]	[0]	[0]	[0]	[0]
minimal	3	0	0	2	0	0	0	0
mild	2	0	0	4	0	0	0	0
moderate	3	0	0	2	0	0	0	0
-hemorrhage	[3]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	2	0	0	0	0	0	0	0
mild	1	0	0	0	0	0	0	0
-hypertrophy, acinar-cell, focal	[2]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	2	0	0	0	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
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TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
PANCREAS (Continued):								
-inflammation, chronic, focal/multifocal	[5]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	3	0	0	2	0	0	0	0
mild	1	0	0	0	0	0	0	0
moderate	1	0	0	1	0	0	0	0
-microgranuloma(s)	1	0	0	0	0	0	0	0
PARATHYROID:								
NO. EXAMINED	27	0	0	25	24	0	0	23
NO. NORMAL	26	0	0	25	24	0	0	23
-ectopic thymic tissue	1	0	0	0	0	0	0	0
PITUITARY:								
NO. EXAMINED	30	0	0	30	28	0	0	23
NO. NORMAL	9	0	0	7	28	0	0	23
-cyst(s), pars distalis	1	0	0	0	0	0	0	0
-hypertrophy/vacuolation, pars distalis	[21]	[0]	[0]	[23]	[0]	[0]	[0]	[0]
minimal	12	0	0	15	0	0	0	0
mild	6	0	0	6	0	0	0	0
moderate	3	0	0	2	0	0	0	0
PROSTATE:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	21	0	0	23				
-prostatitis, interstitial, chronic	[7]	[0]	[0]	[7]				
minimal	5	0	0	6				
mild	2	0	0	1				
-prostatitis, suppurative	[2]	[0]	[0]	[0]				
minimal	1	0	0	0				
mild	1	0	0	0				
RECTUM:								
NO. EXAMINED	29	0	0	29	28	0	0	24
NO. NORMAL	29	0	0	28	25	0	0	23
-parasite(s), lumen	0	0	0	1	3	0	0	1
SALIVARY GLAND:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
SEMINAL VESICLES:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	30	0	0	30				
SKIN (ROUTINE SECTION):								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	20	0	0	18

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 28	2 F 22	3 F 26	4 F 24
SKIN (ROUTINE SECTION) (Continued):								
-dermatitis, chronic	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[2]
minimal	0	0	0	0	1	0	0	1
mild	0	0	0	0	2	0	0	0
moderate	0	0	0	0	0	0	0	1
-hyperplasia/hyperkeratosis, epidermis	[0]	[0]	[0]	[0]	[8]	[0]	[0]	[4]
mild	0	0	0	0	4	0	0	2
moderate	0	0	0	0	4	0	0	2
-necrosis, epidermis, focal	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
mild	0	0	0	0	0	0	0	1
SKIN (GROSS LESION):								
NO. EXAMINED	1	0	0	0	0	0	0	0
NO. NORMAL	0	0	0	0	0	0	0	0
-fibroma	1	0	0	0	0	0	0	0
SPINAL CORD, CERVICAL:								
NO. EXAMINED	30	0	0	30	28	0	0	21
NO. NORMAL	30	0	0	30	28	0	0	21
SPINAL CORD, LUMBAR:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	29	28	0	0	24
-cyst(s), epidermal	0	0	0	1	0	0	0	0
SPINAL CORD, THORACIC:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	30	0	0	30	28	0	0	24
SPLEEN:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	21	0	0	18	27	0	0	24
-cyst(s)	0	0	0	0	1	0	0	0
-hematopoiesis, extramedullary	[9]	[0]	[0]	[10]	[0]	[0]	[0]	[0]
minimal	6	0	0	7	0	0	0	0
mild	3	0	0	3	0	0	0	0
-hemosiderosis	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
mild	0	0	0	2	0	0	0	0
-lymphoma, malignant	0	0	0	1	0	0	0	0
STOMACH:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	22	0	0	26	25	0	0	21
-cystic gland(s), glandular mucosa	0	0	0	0	1	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 28	2 F 22	3 F 26	4 F 24
STOMACH (Continued):								
-dilatation, mucosal glands	[7]	[0]	[0]	[3]	[2]	[0]	[0]	[3]
minimal	4	0	0	2	2	0	0	3
mild	2	0	0	1	0	0	0	0
moderate	1	0	0	0	0	0	0	0
-infiltration, mixed inflammatory cell, submucosa	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-infiltration, polymorphonuclear, submucosa	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
TESTIS:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	30	0	0	30				
THYMUS:								
NO. EXAMINED	30	0	0	30	28	0	0	23
NO. NORMAL	27	0	0	28	23	0	0	19
-atrophy	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[1]
moderate	0	0	0	0	1	0	0	1
-congestion	[3]	[0]	[0]	[2]	[0]	[0]	[0]	[2]
minimal	1	0	0	1	0	0	0	2
mild	2	0	0	1	0	0	0	0
-cyst(s)	0	0	0	0	4	0	0	1
THYROID:								
NO. EXAMINED	30	30	30	30	28	21	26	24
NO. NORMAL	19	18	3	0	20	5	4	0
-Advanced autolysis precludes evaluation	1	0	0	0	0	0	0	0
-adenoma, follicular	1	0	0	0	0	0	0	0
-follicle(s), cystic	2	0	0	0	1	0	1	0
-hyperplasia, C-cell, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-hyperplasia/hypertrophy, follicular epithelium	[2]	[9]	[25]	[30]	[4]	[10]	[20]	[24]
minimal	0	2	1	0	2	4	4	0
mild	1	5	5	0	1	3	8	0
moderate	1	2	11	1	1	3	8	2
marked	0	0	8	29	0	0	0	22
-inflammation, chronic, focal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
-ultimobranchial body/cyst	6	6	4	1	4	8	8	3

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 1 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
P1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	28	22	26	24
TRACHEA:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	24	0	0	23	26	0	0	22
-cystic tracheal glands	0	0	0	1	0	0	0	0
-tracheitis, chronic, focal	[6]	[0]	[0]	[6]	[2]	[0]	[0]	[2]
minimal	6	0	0	6	2	0	0	2
URINARY BLADDER:								
NO. EXAMINED	29	0	0	28	27	0	0	24
NO. NORMAL	28	0	0	28	27	0	0	24
-infiltration, mononuclear-cell, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
UTERUS:								
NO. EXAMINED					28	0	0	24
NO. NORMAL					7	0	0	8
-distention, lumen					[3]	[0]	[0]	[0]
minimal					2	0	0	0
mild					1	0	0	0
-macrophages, pigmented					[21]	[0]	[0]	[16]
minimal					3	0	0	1
mild					5	0	0	2
moderate					12	0	0	13
marked					1	0	0	0
VAGINA:								
NO. EXAMINED					28	0	0	24
NO. NORMAL					28	0	0	24
ZYMBAL'S GLAND:								
NO. EXAMINED	30	0	0	30	28	0	0	24
NO. NORMAL	28	0	0	28	28	0	0	24
-cyst(s)	2	0	0	2	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
ABDOMINAL CAVITY:								
NO. EXAMINED	0	0	0	0	0	0	0	1
NO. NORMAL	0	0	0	0	0	0	0	1
ADRENAL GLANDS:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	24	0	0	23	14	0	0	15
-hypertrophy, cortical, focal	[2]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	2	0	0	0	0	0	0	0
-hypertrophy, zona glomerulosa	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
mild	0	0	0	0	0	0	0	1
-vacuolation, cortical, focal	[4]	[0]	[0]	[7]	[6]	[0]	[0]	[9]
minimal	3	0	0	1	6	0	0	8
mild	0	0	0	4	0	0	0	1
moderate	1	0	0	2	0	0	0	0
AORTA:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
BONE (FEMUR):								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	30	20	0	0	25
-cyst(s), epiphysis	1	0	0	0	0	0	0	0
BONE MARROW (FEMUR):								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
BRAIN:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
CECUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	27	19	0	0	25
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-hyperplasia, lymphoid	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	0	0	2	0	0	0	0
-inflammation, chronic, mucosa	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
mild	1	0	0	0	0	0	0	0
-parasite(s), lumen	0	0	0	0	1	0	0	0
CERVIX:								
NO. EXAMINED					19	0	0	25
NO. NORMAL					19	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 20	2 F 27	3 F 28	4 F 25
COLON:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	19	0	0	23
-parasite(s), lumen	0	0	0	0	1	0	0	2
DUODENUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
EAR(S):								
NO. EXAMINED	0	1	0	0	2	0	1	3
NO. NORMAL	0	0	0	0	0	0	0	0
-chondritis, auricular	[0]	[1]	[0]	[0]	[2]	[0]	[1]	[3]
mild	0	1	0	0	0	0	0	0
moderate	0	0	0	0	0	0	1	1
marked	0	0	0	0	2	0	0	2
EPIDIDYMIDES:								
NO. EXAMINED	30	0	1	30				
NO. NORMAL	21	0	0	26				
-hypospermia	[1]	[0]	[1]	[0]				
marked	1	0	1	0				
-infiltration, mononuclear-cell, focal	[8]	[0]	[0]	[4]				
minimal	8	0	0	4				
ESOPHAGUS:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	30	0	0	30	19	0	0	24
-dilatation	0	0	0	0	0	0	0	1
EYES:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	28	19	0	0	25
-atrophy, retinal, diffuse	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
moderate	0	0	0	0	1	0	0	0
marked	0	0	0	1	0	0	0	0
-keratitis, chronic, focal	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
-mineralization, cornea, focal	[1]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	2	0	0	0	0
HEART:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	19	0	0	18	20	0	0	23

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 20	2 F 27	3 F 28	4 F 25
HEART (Continued):								
-fibrosis, myocardial, focal	[6]	[0]	[0]	[6]	[0]	[0]	[0]	[1]
minimal	6	0	0	3	0	0	0	1
mild	0	0	0	3	0	0	0	0
-hemosiderosis	[5]	[0]	[0]	[6]	[0]	[0]	[0]	[0]
minimal	5	0	0	5	0	0	0	0
mild	0	0	0	1	0	0	0	0
-inflammation, chronic, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
-inflammation, chronic, multifocal	[6]	[0]	[0]	[6]	[0]	[0]	[0]	[1]
minimal	6	0	0	5	0	0	0	1
mild	0	0	0	1	0	0	0	0
ILEUM:								
NO. EXAMINED	30	0	0	29	20	0	0	25
NO. NORMAL	30	0	0	29	20	0	0	25
JEJUNUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	29	20	0	0	25
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
KIDNEYS:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	12	0	0	14	19	0	0	20
-cast(s), granular	0	0	0	1	0	0	0	0
-cyst(s), cortex	1	0	0	2	0	0	0	1
-cyst(s), medulla	1	0	0	0	1	0	0	0
-degeneration/basophilia, cortical tubules, focal	[3]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	3	0	0	5	0	0	0	0
-dilatation, cortical tubules	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	0	0	2	0	0	0	0
-dilatation, medullary tubules	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-dilatation, pelvis	[2]	[0]	[0]	[5]	[1]	[0]	[0]	[0]
mild	2	0	0	5	1	0	0	0
-dilatation, tubules, papilla	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 20	2 F 27	3 F 28	4 F 25
KIDNEYS (Continued):								
-glomerulonephritis, chronic	[2]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	1	0	0	0	0
mild	1	0	0	1	0	0	0	0
-hyaline droplets, cortical tubules	[9]	[0]	[0]	[7]	[0]	[0]	[0]	[0]
minimal	3	0	0	0	0	0	0	0
mild	5	0	0	3	0	0	0	0
moderate	1	0	0	4	0	0	0	0
-infiltration, mononuclear-cell, focal/multifocal	[2]	[0]	[0]	[5]	[0]	[0]	[0]	[1]
minimal	2	0	0	5	0	0	0	1
-mineralization, multifocal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	1	0	0	0	0	0	0	1
-mineralization, pelvis	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[2]
minimal	0	0	0	0	0	0	0	2
LIVER:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	13	0	0	16	13	0	0	22
-hematopoiesis, extramedullary	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[0]
minimal	0	0	0	1	1	0	0	0
-inflammation, chronic, focal/multifocal	[17]	[0]	[0]	[13]	[6]	[0]	[0]	[3]
minimal	17	0	0	12	6	0	0	3
mild	0	0	0	1	0	0	0	0
LUNG:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	18	0	0	23	5	0	0	13
-infiltration, eosinophilic, perivascular and peribronchial	[0]	[0]	[0]	[0]	[2]	[0]	[0]	[1]
minimal	0	0	0	0	2	0	0	1
-inflammation, interstitial, acute, focal	[0]	[0]	[0]	[1]	[1]	[0]	[0]	[3]
minimal	0	0	0	1	1	0	0	3
-inflammation, interstitial, chronic, focal	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[1]
minimal	0	0	0	0	0	0	0	1
-macrophages, alveoli, focal	[10]	[0]	[0]	[6]	[12]	[0]	[0]	[9]
minimal	8	0	0	5	11	0	0	8
mild	2	0	0	1	1	0	0	1
-proliferation, lymphoid, peribronchial/perivascular	[3]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	3	0	0	2	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
LYMPH NODE, MANDIBULAR:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	16	0	0	16	9	0	0	10
-hyperplasia, lymphocytic/plasmacytic	[14]	[0]	[0]	[14]	[11]	[0]	[0]	[15]
minimal	7	0	0	6	3	0	0	8
mild	3	0	0	3	2	0	0	5
moderate	4	0	0	4	6	0	0	2
marked	0	0	0	1	0	0	0	0
LYMPH NODE, MESENTERIC:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	28	14	0	0	18
-congestion	[0]	[0]	[0]	[0]	[2]	[0]	[0]	[0]
minimal	0	0	0	0	2	0	0	0
-histiocytosis	[0]	[0]	[0]	[2]	[4]	[0]	[0]	[7]
minimal	0	0	0	1	2	0	0	3
mild	0	0	0	1	2	0	0	4
-hyperplasia, lymphoid	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
mild	0	0	0	0	1	0	0	0
MAMMARY GLAND:								
NO. EXAMINED	22	0	0	24	20	0	0	24
NO. NORMAL	22	0	0	24	20	0	0	23
-cystic gland/duct	0	0	0	0	0	0	0	1
MUSCLE, SKELETAL:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	30	20	0	0	25
-inflammation, chronic, focal	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
NERVE, SCIATIC:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
OVARIES:								
NO. EXAMINED					20	0	0	25
NO. NORMAL					19	0	0	25
-cyst(s)					1	0	0	0
PANCREAS:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	16	0	0	18	20	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
PANCREAS (Continued):								
-atrophy, acinar, focal	[2]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	1	0	0	1	0	0	0	0
mild	1	0	0	1	0	0	0	0
-fibrosis, islet, focal	[9]	[0]	[0]	[5]	[0]	[0]	[0]	[0]
minimal	5	0	0	4	0	0	0	0
mild	4	0	0	1	0	0	0	0
-hemorrhage	[3]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	2	0	0	2	0	0	0	0
mild	1	0	0	0	0	0	0	0
-hypertrophy, acinar-cell, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-inflammation, acute, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-inflammation, chronic, focal/multifocal	[5]	[0]	[0]	[6]	[0]	[0]	[0]	[0]
minimal	5	0	0	4	0	0	0	0
mild	0	0	0	2	0	0	0	0
-inflammation, subacute, focal	[3]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	3	0	0	0	0	0	0	0
PARATHYROID:								
NO. EXAMINED	25	0	0	27	19	0	0	25
NO. NORMAL	25	0	0	27	19	0	0	25
PITUITARY:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	17	0	0	20	20	0	0	25
-cyst(s), pars distalis	1	0	0	0	0	0	0	0
-hyperplasia, pars distalis, focal	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
minimal	0	0	0	1	0	0	0	0
-hypertrophy/vacuolation, pars distalis	[13]	[0]	[0]	[9]	[0]	[0]	[0]	[0]
minimal	10	0	0	4	0	0	0	0
mild	1	0	0	4	0	0	0	0
moderate	2	0	0	1	0	0	0	0
PROSTATE:								
NO. EXAMINED	30	0	1	30				
NO. NORMAL	21	0	0	24				

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 20	2 F 27	3 F 28	4 F 25
PROSTATE (Continued):								
-prostatitis, interstitial, chronic	[9]	[0]	[0]	[6]				
minimal	5	0	0	5				
mild	4	0	0	0				
moderate	0	0	0	1				
-prostatitis, suppurative	[0]	[0]	[1]	[0]				
marked	0	0	1	0				
RECTUM:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	16	0	0	25
-parasite(s), lumen	0	0	0	0	4	0	0	0
SALIVARY GLAND:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
SEMINAL VESICLES:								
NO. EXAMINED	30	0	0	30				
NO. NORMAL	30	0	0	30				
SKIN (ROUTINE SECTION):								
NO. EXAMINED	29	0	0	29	20	0	0	24
NO. NORMAL	28	0	0	29	14	0	0	16
-dermatitis, chronic, focal	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[0]
minimal	0	0	0	0	1	0	0	0
mild	0	0	0	0	1	0	0	0
moderate	0	0	0	0	1	0	0	0
-hyperplasia, epidermis	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
minimal	1	0	0	0	0	0	0	0
-hyperplasia/hyperkeratosis, epidermis	[0]	[0]	[0]	[0]	[4]	[0]	[0]	[8]
minimal	0	0	0	0	3	0	0	2
mild	0	0	0	0	1	0	0	5
moderate	0	0	0	0	0	0	0	1
-necrosis, focal	[0]	[0]	[0]	[0]	[1]	[0]	[0]	[0]
moderate	0	0	0	0	1	0	0	0
SKIN (GROSS LESION):								
NO. EXAMINED	0	0	1	0	0	0	1	0
NO. NORMAL	0	0	0	0	0	0	0	0
-dermatitis, chronic, focal	[0]	[0]	[1]	[0]	[0]	[0]	[1]	[0]
minimal	0	0	0	0	0	0	1	0
moderate	0	0	1	0	0	0	0	0
SPINAL CORD, CERVICAL:								
NO. EXAMINED	29	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	30	20	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group:	1	2	3	4	1	2	3	4
Sex:	M	M	M	M	F	F	F	F
Number of Animals/Group:	30	30	30	30	20	27	28	25
SPINAL CORD, LUMBAR:								
NO. EXAMINED	30	0	0	29	20	0	0	25
NO. NORMAL	30	0	0	29	20	0	0	25
SPINAL CORD, THORACIC:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	30	0	0	30	20	0	0	25
SPLEEN:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	22	0	0	20	16	0	0	23
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-hematopoiesis, extramedullary	[1]	[0]	[0]	[3]	[0]	[0]	[0]	[0]
minimal	1	0	0	3	0	0	0	0
-hemosiderosis	[8]	[0]	[0]	[6]	[4]	[0]	[0]	[2]
minimal	2	0	0	2	1	0	0	2
mild	5	0	0	3	3	0	0	0
moderate	1	0	0	1	0	0	0	0
STOMACH:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	24	0	0	24	17	0	0	18
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-cyst(s), squamous	1	0	0	0	0	0	0	0
-dilatation, mucosal glands	[5]	[0]	[0]	[5]	[3]	[0]	[0]	[7]
minimal	3	0	0	3	1	0	0	7
mild	2	0	0	2	2	0	0	0
TESTIS:								
NO. EXAMINED	30	0	1	30				
NO. NORMAL	29	0	0	30				
-atrophy, diffuse	[1]	[0]	[1]	[0]				
marked	1	0	1	0				
THYMUS:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	24	0	0	21	14	0	0	20
-atrophy	[0]	[0]	[0]	[0]	[3]	[0]	[0]	[2]
minimal	0	0	0	0	0	0	0	1
mild	0	0	0	0	3	0	0	1
-congestion	[6]	[0]	[0]	[9]	[0]	[0]	[0]	[1]
minimal	4	0	0	4	0	0	0	1
mild	1	0	0	4	0	0	0	0
moderate	1	0	0	1	0	0	0	0
-cyst(s)	0	0	0	0	2	0	0	2

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 20	2 F 27	3 F 28	4 F 25
THYROID:								
NO. EXAMINED	30	30	30	30	20	27	27	25
NO. NORMAL	21	14	7	0	5	16	8	0
-Advanced autolysis precludes evaluation	0	0	0	1	0	0	0	0
-adenoma, follicular	0	0	0	1	0	0	0	0
-hyperplasia, follicular, focal/nodular	[0]	[0]	[0]	[1]	[0]	[0]	[0]	[0]
marked	0	0	0	1	0	0	0	0
-hyperplasia/hypertrophy, follicular epithelium	[5]	[8]	[19]	[26]	[6]	[6]	[13]	[24]
minimal	0	0	3	0	4	5	6	1
mild	3	4	5	0	2	1	6	2
moderate	2	4	5	3	0	0	1	10
marked	0	0	6	23	0	0	0	11
-ultimobranchial body/cyst	7	8	6	6	13	9	15	8
TRACHEA:								
NO. EXAMINED	30	0	0	30	19	0	0	25
NO. NORMAL	27	0	0	28	18	0	0	24
-tracheitis, chronic, focal	[3]	[0]	[0]	[2]	[1]	[0]	[0]	[1]
minimal	3	0	0	2	1	0	0	1
URINARY BLADDER:								
NO. EXAMINED	30	0	0	29	20	0	0	25
NO. NORMAL	30	0	0	27	20	0	0	25
-infiltration, mononuclear-cell, focal	[0]	[0]	[0]	[2]	[0]	[0]	[0]	[0]
minimal	0	0	0	2	0	0	0	0
UTERUS:								
NO. EXAMINED					20	0	0	25
NO. NORMAL					1	0	0	2
-deciduoma					1	0	0	0
-distention, lumen					[3]	[0]	[0]	[3]
minimal					0	0	0	1
mild					2	0	0	1
moderate					1	0	0	1
-macrophages, pigmented					[17]	[0]	[0]	[22]
minimal					1	0	0	3
mild					7	0	0	8
moderate					9	0	0	11
VAGINA:								
NO. EXAMINED					19	0	0	25
NO. NORMAL					19	0	0	25
ZYMBAL'S GLAND:								
NO. EXAMINED	30	0	0	30	20	0	0	25
NO. NORMAL	29	0	0	29	20	0	0	25

[ ] = Total incidence of specified lesion, all grades.

ORAL (DRINKING WATER) TWO-GENERATION (ONE LITTER PER GENERATION)
REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS
PROTOCOL 1416-001

TABLE 2 (Continued)

Incidence and Degree of Severity of Histomorphologic Observations
F1 Generation

Dose Group: Sex: Number of Animals/Group:	1 M 30	2 M 30	3 M 30	4 M 30	1 F 20	2 F 27	3 F 28	4 F 25
ZYMBAL'S GLAND (Continued):								
-cyst(s)	1	0	0	1	0	0	0	0

[ ] = Total incidence of specified lesion, all grades.

February 8, 1999 EPA Assessment Submission

Attachment #4
Review of 14-Day Exposure on Antibody Response to SRBC in Mice

A. SRBC Specific Serum IgM Antibody Response (Keil,1999b)
B. Preliminary EPA analysis (Smialowicz, 1999b)

ATTENTION PANEL MEMBER(S):

KIMBER WHITE

Immunotoxicity Studies at Medical University of South Carolina

(14-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"C, G, I, J, T, K"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured. Supplementary experiments were needed to acquire additional serum samples for hormone analysis or to repeat the NK assay.
"U, V"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and were challenged with listeria to assess **delayed type hypersensitivity**.
"H, F, M"	B6C3F1 female mice were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
SRBC Experiments	B6C3F1 female mice (two experiments of 30 mice each) were exposed to **90 days of AP** (0, 0.1, 3.0, or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 75, bled on day 79 to determine specific IgM antibody levels, and bled on day 90 to determine specific IgG antibody levels.

Immunotoxicity Studies at Medical University of South Carolina
(90-Day Data)

Unique Experiment "Letter" Designation	Experimental Description
"A, D, N"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of immune, hematological, or thyroid parameters were measured. In experiments "A" and "D", thyroid histopathology was performed. Experiment "N" included a variety of other parameters: macrophage phagocytosis and nitrite production, NKh, assay organ weights and cellularities, flow cytometry, and serum for hormone analysis.
"B, E"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and a variety of **immune, hematological, or thyroid parameters** were measured.
"P"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with P815 cells and **CTL activity** was assessed.
"Q"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with **B16F10 melanomas** on day 76.
"L"	B6C3F1 female mice were exposed to **90 days of AP** (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water and mice were challenged with ***Listeria monocytogenes*** on day 86.
SRBC Experiments	B6C3F1 Female mice (1 experiment of 30 mice) were exposed to **14 days of AP** (0, 0.1, 1.0, 3.0 or 30 mg/kg-day) via drinking water. Mice were challenged with SRBC on day 9 and bled on day 14 to determine specific IgM antibody levels.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Experimental Toxicology Division
.Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

DATE: February 5, 1999

FROM: Ralph J. Smialowicz (MD-92) R.J. Smialowicz

TO: Annie Jarabek (MD-52)
National Center for Environmental Assessment

SUBJECT: Review of 14-Day Ammonium Perchlorate Exposure on the Antibody Response to SRBC in Mice

Results of a 14-day study were received on February 4, 1999 for review. In this study, B6C3F1 female mice, 6 mice per group, were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 14 days. Mice where immunized intravenously with SRBC on day 9. Serum was collected on day 14 (5 days post-immunization)and the SRBC-specific IgM antibody levels were determined using an enzyme-linked immunosorbent assay (ELISA). The ELISA data were analyzed "as described by L. Temple of the Medical College of Virginia", and expressed as the O.D. 50. Analysis of the ELISA data indicated that the IgM titers were not affected in mice exposed to ammonium perchlorate for 14 days compared to controls.

SRBC Specific Serum IgM Determination after Exposure to Ammonium Perchlorate for 14 Days

Submitted by Deborah Keil, PhD
Medical University of South Carolina
February 4, 1999

Animals and Ammonium Perchlorate Exposure: B6C3F1 female mice aged 8-10 weeks were exposed to ammonium perchlorate (AP) (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 14 days. A total of 30 mice with 6 animals per treatment group were used to determine specific IgM levels after immunization with sRBC. Animals were housed in an AAALAC accredited facility and provided water (with and without AP) and mouse chow ad libidum.

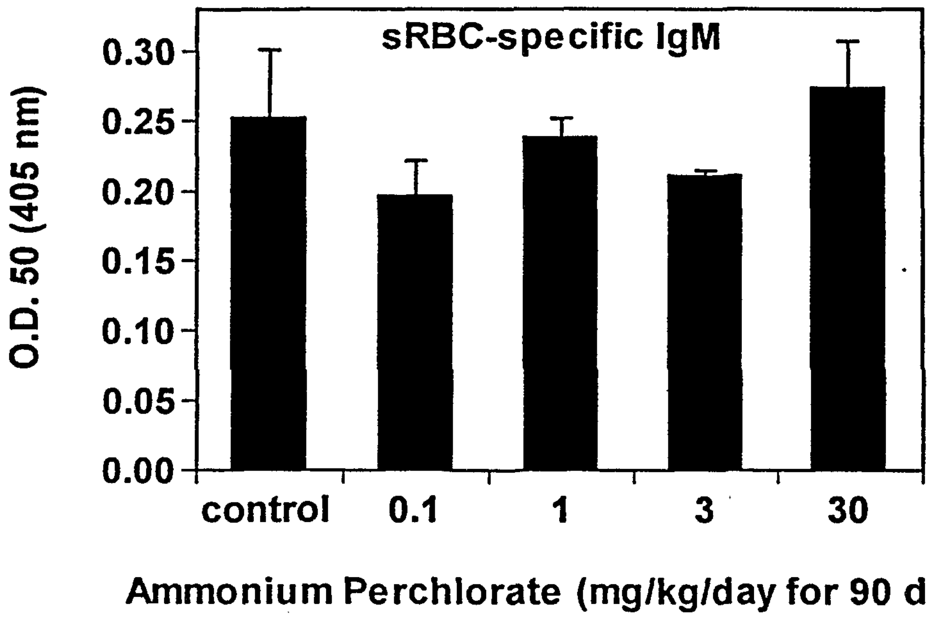
Immunization: Mice were immunized with sheep red blood cells (sRBC) ($1x10^8$ total cells) by intravenous tail injection on day 9. Serum was collected on day 14 (5 days post challenge) to determine specific IgM antibody levels, respectively. A semi-quantitative ELISA detected levels of specific IgM sRBC antibody in serially diluted serum (1:10, 1:20, 1:40, 1:80, 1:160).

Optimization of the ELISA: Optimization of the ELISA was performed prior to testing the serum samples to establish the appropriate titer of sRBC membrane coating antigen (1μg/ml) and the secondary antibody dilution (1:5,000 for IgM). In addition, pooled serum samples from controls were used in the optimization. Controls for non-specific binding were included and were approximately 0.070 O.D. (405 nm) in both the optimization and testing ELISAs.

Data Analysis:
Analysis of sRBC specific IgG serum titers were analyzed as described by L. Temple of the Medical College of Virginia. The average absorbance unit values of the replicates for each dilution of the test serum were calculated. Background in the ELISA was subtracted from these values. Five consecutive average absorbance values versus log base 2 of the dilution of the serum were plotted. The best-fit linear line was calculated in an Excel spreadsheet by determining the value for the slope and intercept. Log base 2 of the titer was considered the independent variable and O.D. was considered the dependent variable. In this experiment, the absorbance at the mid-point of the 5 serial dilutions was 1:40. Using the equation for the best-fit line, the O.D. 50 (absorbance at mid-point 1:40) was calculated for each animal.

Results:
No significant differences in specific sRBC IgM antibody were observed in any of the AP treatment groups as compared to controls. This is consistent with results obtained after 90-day exposure to AP. The results were expressed as the O.D.50 and an analysis of variance was performed using Tukey's pairwise comparisons ($p<0.05$). Refer to graphs and statistical analysis that have been included in this report.

Serum IgM Levels after sRBC Challenge During a 14-Day Exposure to Ammonium Perchlorate



Adult B6C3F1 female mice were exposed to ammonium perchlorate (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) via drinking water for 14 days. On day 9 (5 days before serum was collected), animals were immunized by i.v. tail injection with sRBC (1 x 10^8 cells). Detection of specific IgM was performed using an ELISA based on a protocol by L. Temple at the Medical College of Virginia. The O.D. 50 was determined for IgM. The above graph represents the means and standard errors of 6 animals per group for a total of 30 mice. No significant differences were observed in any of the treatment groups as compared to controls using analysis of variance and Tukey's pairwise comparisons ($p<0.05$).

Statistics

The calculated O.D. 50 for each of the treatment groups was compared to controls ($p<0.05$). A total of 30 serum samples from independently challenged mice (6 mice per group) were analyzed for both IgM levels.

Descriptive Statistics

Variable	N	Mean	Median	TrMean	StDev	SE Mean
control	6	0.2512	0.2275	0.2512	0.1216	0.0496
0.1	6	0.1958	0.1965	0.1958	0.0624	0.0255
1.0	6	0.2373	0.2285	0.2373	0.0359	0.0147
3.0	6	0.21067	0.21250	0.21067	0.01073	0.00438
30	6	0.2730	0.2850	0.2730	0.0832	0.0340

Variable	Minimum	Maximum	Q1	Q3
control	0.1250	0.4790	0.1707	0.3162
0.1	0.1250	0.2860	0.1302	0.2508
1.0	0.1980	0.2840	0.2063	0.2780
3.0	0.19800	0.22400	0.19875	0.21950
30	0.1760	0.3620	0.1827	0.3493

One-way Analysis of Variance

Analysis of Variance for IgM 14d

Source	DF	SS	MS	F	P
treatmen	4	0.02296	0.00574	1.06	0.395
Error	25	0.13499	0.00540		
Total	29	0.15795			

```
                                     Individual 95% CIs For Mean
                                     Based on Pooled StDev
Level       N      Mean     StDev    --------+---------+---------+--------
 0.0        6   0.25117   0.12159              (---------*---------)
 0.1        6   0.19583   0.06237    (----------*---------)
 1.0        6   0.23733   0.03590            (----------*---------)
 3.0        6   0.21067   0.01073       (---------*---------)
30.0        6   0.27300   0.08318                 (----------*---------)
                                     --------+---------+---------+--------
Pooled StDev =  0.07348                    0.180     0.240     0.300
```

Tukey's pairwise comparisons

Family error rate = 0.0500
Individual error rate = 0.00706

Critical value = 4.15

Intervals for (column level mean) - (row level mean)

	0.0	0.1	1.0	3.0
0.1	-0.06916 0.17983			
1.0	-0.11066 0.13833	-0.16599 0.08299		
3.0	-0.08399 0.16499	-0.13933 0.10966	-0.09783 0.15116	
30.0	-0.14633 0.10266	-0.20166 0.04733	-0.16016 0.08883	-0.18683 0.06216

February 8, 1999 EPA Assessment Submission

Attachment #5
Correlations of Hormone and Histopathology Data from Caldwell et al. (1995), Neurodevelopmental Study (Argus, 1998a), and Subchronic Study (Springborn, 1998) with Percent Iodide Inhibition (Meyer, 1998; Channel, 1999)

A. Iodide Inhibition 14-day study (Channel, 1999)
B. Preliminary EPA analysis (Geller, 1999d)

ATTENTION PANEL MEMBER(S):

MEL ANDERSEN

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HEALTH AND ENVIRONMENTAL EFFECTS
RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

Neurotoxicology Division, MD-74B

MEMORANDUM

Date: 8 February 1999

Subject: Correlations of Hormone and Histopathology Data from Caldwell et. al. (1995), Neurobehavioral Developmental (Argus, 1998a), and Subchronic (Springborn, 1998) Studies with Percent Iodide Uptake Inhibition (Meyer, 1998).

From: Andrew M. Geller
Neurotoxicology Division, MD-74B
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

Correlations and linear regressions were run, as requested, to relate estimated administered dose or iodide uptake inhibition associated with a particular dose of ammonium perchlorate to thyroxine (T4), 3,5,3'-triiodothyronine (T3), and thyrotrophin (TSH) in the Caldwell 14-day Study, the Neurobehavioral Developmental Study, and the Subchronic Study. These were run to begin to evaluate the efficacy of using the inhibition of iodide uptake by the thyroid as an internal dosimeter rather than using estimated administered dose.

The results of two studies were considered to establish the relationship of administered dose of ammonium perchlorate and iodide uptake inhibition. Meyer (1998) measured iodide uptake inhibition 9 hours after the acute i.v. administration of a single dose of ammonium perchlorate was used in these calculations. This study showed a dose-related decrease in iodide uptake by the thyroid. Channel (1999) measured iodide uptake inhibition in adult male animals that had been dosed for 14 days with ammonium perchlorate in the drinking water. This study did not show a systematic change in iodide uptake with administered dose of perchlorate.

Caldwell 14 Day study: Note that these correlations are run using only 4 of 9 doses (0, 0.1, 1.0, 3.0 mg/kg/day) used in the study, since not all study doses were included in Meyer (1998) and Channel (1999).

Correlations with Meyer (1998): Percentage of iodide uptake inhibition correlated more highly with T4 and T3 levels than did estimated administered dose. The correlation of TSH with administered dose or iodide uptake inhibition was approximately the same (Table 1A).

Correlations with Channel (1999): Correlations with the non-monotonic uptake data (Table 1B) were much worse than those with administered dose or with uptake as established in the Meyer (1998) study.

Neurobehavioral Developmental study: Note that these correlations are run using only 4 of 5 doses (0. 0.1, 1.0, 3.0 mg/kg/day) used in the study, since not all study doses were included in Meyer (1998) and Channel (1999).

Correlations with Meyer (1998), Post-natal day 5 (PND5) pups: Correlations of T3 and T4 with administered dose or iodide uptake inhibition were significant. The correlation of TSH with iodide uptake inhibition was marginally significant. Correlations of administered dose or iodide uptake inhibition with T4 and TSH were approximately equal. T3 correlated more highly with administered dose than iodide uptake inhibition (Table 2A).

Correlations with Channel (1999): Correlations of hormone levels with iodide uptake as estimated by the results reported in Channel (1999) were worse than those with administered dose or the iodide uptake numbers of Meyer (1998).

Subchronic Study: Note that these correlations are run using only 3 of 6 doses (0, 0.01, 1.0 mg/kg/day) used in the study, since not all study doses were included in Meyer (1998) and Channel (1999).

Correlations with Meyer (1998):

14 day timepoint: Correlations of T3 and TSH with administered dose or iodide uptake inhibition were significant. Correlations with T4 were not. Correlations of T4, T3 and TSH were

marginally higher with iodide uptake inhibition than with administered dose (Table 3).

90 day timepoint: Correlations of T4, T3 and TSH were significant with both administered dose and iodide uptake inhibition. Correlations were marginally higher with iodide uptake inhibition than with administered dose (Table 4).

Correlations with Channel (1999):

At both 14 and 90 day timepoints, the correlations with the estimates of iodide uptake from the 14 day drinking water administration of perchlorate were worse than from the other measures of dosimetry discussed in this memo and were of opposite sign.

Discussion: The correlations run in this memo were done with subsets of the data from each of the relevant studies. These subsets were composed of animals from the dose groups in the studies that were identical to those considered in the iodide uptake studies. For future consideration, it may prove useful to fit the monotonic data of Meyer (1998) with an analytic function that would allow for the interpolation of iodide uptake values at all study doses. The data included in Channel (1999) are more problematic. Previous data on absorption, distribution, metabolism, and elimination do not suggest a U-shaped relationship between perchlorate dose and iodide uptake by the thyroid. Further work is necessary to elaborate differences between injected and oral dosing.

Table 3 -- Subchronic Study – Correlations of hormone levels from 14 day time point with estimated administered dose of ammonium perchlorate and with % iodide uptake inhibition.
A. Uptake inhibiton derived from 9 hour timepoint of Meyer (1998).

	T4	T3	TSH
Administered Dose	-0.11 0.44	-0.49 0.0002	0.44 0.001
% Iodide Uptake Inhibition	-0.14 0.334	-0.52 0.0001	0.45 0.0007

B. Uptake inhibition derived from 2 hour timepoint of Channel (1999).

	T4	T3	TSH
Administered Dose	-0.11 0.44	-0.49 0.0002	0.44 0.001
% Iodide Uptake Inhibition	0.04 0.77	0.38 0.005	-0.38 0.005

Table 4 -- Subchronic Study – Correlations of hormone levels from 90 day time point with estimated administered dose of ammonium perchlorate and with % iodide uptake inhibition

A. Uptake inhibition derived from 9 hour timepoint of Meyer (1998).

	T4	T3	TSH
Administered Dose	-0.62 0.0001	-0.72 0.0001	0.47 0.0002
% Iodide Uptake Inhibition	-0.69 0.0001	-0.77 0.0001	0.48 0.0001

B. Uptake inhibition derived from 2 hour timepoint of Channel (1999).

	T4	T3	TSH
Administered Dose	-0.62 0.0001	-0.72 0.0001	0.47 0.0002
% Iodide Uptake Inhibition	0.42 0.001	0.53 0.001	-0.398 0.002

1 T4, T3, TSH, and Follicle Hyperplasia and follicular lumen 1
area data from Caldwell 14-day perchlorate study with PK internal dose approximation from Meyer, 1998
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OBS	ID	DOSE	SEX	FH	FL	T4	T3	TSH	DCORR	INTDOSE
1	68	0.00	F	1	1	4.49	124.33	10.92	0.0	0
2	71	0.00	F	1	1	4.50	138.36	10.71	0.0	0
3	86	0.00	F	0	1	5.36	127.89	12.01	0.0	0
4	90	0.00	F	0	1	5.36	133.14	11.40	0.0	0
5	95	0.00	F	0	0	4.94	134.17	10.96	0.0	0
6	100	0.00	F	0	0	5.33	113.17	11.49	0.0	0
7	51	1.25	F	0	0	4.41	93.91	11.96	0.1	29
8	55	1.25	F	.	.	4.37	75.70	11.71	0.1	29
9	58	1.25	F	0	1	4.47	84.17	14.74	0.1	29
10	67	1.25	F	1	0	4.37	85.04	13.42	0.1	29
11	73	1.25	F	0	1	4.13	86.66	13.68	0.1	29
12	84	1.25	F	0	0	4.69	82.11	12.81	0.1	29
13	60	12.50	F	1	1	3.75	94.00	15.45	1.0	55
14	66	12.50	F	1	1	3.84	79.42	12.54	1.0	55
15	70	12.50	F	0	1	4.11	76.16	16.42	1.0	55
16	77	12.50	F	0	0	3.97	77.21	15.93	1.0	55
17	92	12.50	F	1	1	4.11	78.67	16.43	1.0	55
18	99	12.50	F	1	1	3.92	78.61	15.37	1.0	55
19	59	25.00	F	2	1	3.75	73.91	17.17	3.0	82
20	64	25.00	F	1	1	3.96	85.00	17.21	3.0	82
21	76	25.00	F	1	2	3.80	73.47	17.33	3.0	82
22	80	25.00	F	1	1	3.75	74.16	17.55	3.0	82
23	87	25.00	F	2	2	4.11	88.61	16.77	3.0	82
24	94	25.00	F	1	1	3.76	80.65	18.29	3.0	82
25	11	0.00	M	0	1	5.19	139.85	14.24	0.0	0
26	15	0.00	M	1	1	5.12	141.54	13.76	0.0	0
27	24	0.00	M	0	0	5.16	136.13	14.68	0.0	0
28	39	0.00	M	0	0	4.98	110.68	13.88	0.0	0
29	44	0.00	M	0	1	5.44	129.32	16.69	0.0	0
30	47	0.00	M	1	0	4.85	139.67	13.59	0.0	0
31	1	1.25	M	1	0	4.86	132.68	14.87	0.1	29
32	12	1.25	M	0	1	4.79	127.29	15.03	0.1	29
33	14	1.25	M	1	0	4.83	137.89	12.32	0.1	29
34	19	1.25	M	1	1	4.73	126.69	15.85	0.1	29
35	25	1.25	M	1	1	4.70	106.41	16.70	0.1	29
36	27	1.25	M	1	1	4.91	113.15	15.35	0.1	29
37	17	12.50	M	1	2	4.35	99.33	22.88	1.0	55
38	20	12.50	M	1	1	4.34	96.77	21.74	1.0	55
39	26	12.50	M	0	1	3.92	85.19	18.79	1.0	55
40	38	12.50	M	1	0	4.53	98.02	18.45	1.0	55
41	41	12.50	M	2	2	4.29	81.90	19.92	1.0	55
42	45	12.50	M	1	1	4.49	81.55	19.72	1.0	55
43	4	25.00	M	1	2	4.06	85.83	32.66	3.0	82
44	7	25.00	M	2	1	4.26	70.56	28.76	3.0	82
45	16	25.00	M	2	2	4.16	77.17	30.41	3.0	82

	DOSE	DCORR	INTDOSE	T4	T3	TSH	FH	FL
	0.0001	0.0001	0.0001	0.0010	0.0180	0.0011	0.0	0.0006
	47	47	47	47	47	47	47	47
FL	0.53089	0.51594	0.49650	-0.38990	-0.34003	0.48478	0.48237	1.00000
	0.0001	0.0002	0.0004	0.0067	0.0194	0.0006	0.0006	0.0
	47	47	47	47	47	47	47	47

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 3

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Correlation Analysis

Spearman Correlation Coefficients / Prob > |R| under Ho: Rho=0 / Number of Observations

	DOSE	DCORR	INTDOSE	T4	T3	TSH	FH	FL
DOSE	1.00000	1.00000	1.00000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
DCORR	1.00000	1.00000	1.00000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0001	0.0	0.0001	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
INTDOSE	1.00000	1.00000	1.00000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0001	0.0001	0.0	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
T4	-0.83826	-0.83826	-0.83826	1.00000	0.77215	-0.50928	-0.43423	-0.41697
	0.0001	0.0001	0.0001	0.0	0.0001	0.0002	0.0023	0.0035
	48	48	48	48	48	48	47	47
T3	-0.81379	-0.81379	-0.81379	0.77215	1.00000	-0.56564	-0.35229	-0.33194
	0.0001	0.0001	0.0001	0.0001	0.0	0.0001	0.0152	0.0226
	48	48	48	48	48	48	47	47
TSH	0.81243	0.81243	0.81243	-0.50928	-0.56564	1.00000	0.50107	0.54035
	0.0001	0.0001	0.0001	0.0002	0.0001	0.0	0.0003	0.0001
	48	48	48	48	48	48	47	47
FH	0.59016	0.59016	0.59016	-0.43423	-0.35229	0.50107	1.00000	0.45934
	0.0001	0.0001	0.0001	0.0023	0.0152	0.0003	0.0	0.0012
	47	47	47	47	47	47	47	47
FL	0.49760	0.49760	0.49760	-0.41697	-0.33194	0.54035	0.45934	1.00000
	0.0004	0.0004	0.0004	0.0035	0.0226	0.0001	0.0012	0.0
	47	47	47	47	47	47	47	47

1 CALDWELL STUDY, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 4

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Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	780.35450	780.35450	46.011	0.0001
Error	45	763.20148	16.96003		
C Total	46	1543.55598			

Root MSE	4.11826	R-square	0.5056
Dep Mean	17.24298	Adj R-sq	0.4946
C.V.	23.88367		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	11.696723	1.01459484	11.528	0.0001
INTDOSE	1	0.132794	0.01957694	6.783	0.0001

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6.40591	6.40591	23.983	0.0001
Error	45	12.01962	0.26710		
C Total	46	18.42553			

Root MSE	0.51682	R-square	0.3477
Dep Mean	0.76596	Adj R-sq	0.3332
C.V.	67.47370		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.263448	0.12732641	2.069	0.0443
INTDOSE	1	0.012032	0.00245680	4.897	0.0001

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.4900	5.0114	0.067	4.8756	5.1471	-0.5214
2	4.5000	5.0114	0.067	4.8756	5.1471	-0.5114
3	5.3600	5.0114	0.067	4.8756	5.1471	0.3486
4	5.3600	5.0114	0.067	4.8756	5.1471	0.3486
5	4.9400	5.0114	0.067	4.8756	5.1471	-0.0714
6	5.3300	5.0114	0.067	4.8756	5.1471	0.3186
7	4.4100	4.6268	0.043	4.5397	4.7138	-0.2168
8	4.3700	4.6268	0.043	4.5397	4.7138	-0.2568
9	4.4700	4.6268	0.043	4.5397	4.7138	-0.1568
10	4.3700	4.6268	0.043	4.5397	4.7138	-0.2568
11	4.1300	4.6268	0.043	4.5397	4.7138	-0.4968
12	4.6900	4.6268	0.043	4.5397	4.7138	0.0632
13	3.7500	4.2819	0.043	4.1944	4.3694	-0.5319
14	3.8400	4.2819	0.043	4.1944	4.3694	-0.4419
15	4.1100	4.2819	0.043	4.1944	4.3694	-0.1719
16	3.9700	4.2819	0.043	4.1944	4.3694	-0.3119
17	4.1100	4.2819	0.043	4.1944	4.3694	-0.1719
18	3.9200	4.2819	0.043	4.1944	4.3694	-0.3619
19	3.7500	3.9238	0.066	3.7913	4.0564	-0.1738
20	3.9600	3.9238	0.066	3.7913	4.0564	0.0362
21	3.8000	3.9238	0.066	3.7913	4.0564	-0.1238
22	3.7500	3.9238	0.066	3.7913	4.0564	-0.1738
23	4.1100	3.9238	0.066	3.7913	4.0564	0.1862
24	3.7600	3.9238	0.066	3.7913	4.0564	-0.1638
25	5.1900	5.0114	0.067	4.8756	5.1471	0.1786
26	5.1200	5.0114	0.067	4.8756	5.1471	0.1086
27	5.1600	5.0114	0.067	4.8756	5.1471	0.1486
28	4.9800	5.0114	0.067	4.8756	5.1471	-0.0314
29	5.4400	5.0114	0.067	4.8756	5.1471	0.4286
30	4.8500	5.0114	0.067	4.8756	5.1471	-0.1614
31	4.8600	4.6268	0.043	4.5397	4.7138	0.2332
32	4.7900	4.6268	0.043	4.5397	4.7138	0.1632
33	4.8300	4.6268	0.043	4.5397	4.7138	0.2032
34	4.7300	4.6268	0.043	4.5397	4.7138	0.1032
35	4.7000	4.6268	0.043	4.5397	4.7138	0.0732
36	4.9100	4.6268	0.043	4.5397	4.7138	0.2832
37	4.3500	4.2819	0.043	4.1944	4.3694	0.0681
38	4.3400	4.2819	0.043	4.1944	4.3694	0.0581
39	3.9200	4.2819	0.043	4.1944	4.3694	-0.3619
40	4.5300	4.2819	0.043	4.1944	4.3694	0.2481
41	4.2900	4.2819	0.043	4.1944	4.3694	0.00808
42	4.4900	4.2819	0.043	4.1944	4.3694	0.2081
43	4.0600	3.9238	0.066	3.7913	4.0564	0.1362

34	126.7	108.5	2.083	104.3	112.7	18.1718
35	106.4	108.5	2.083	104.3	112.7	-2.1082
36	113.2	108.5	2.083	104.3	112.7	4.6318
37	99.3300	91.1422	2.094	86.9243	95.3601	8.1878
38	96.7700	91.1422	2.094	86.9243	95.3601	5.6278
39	85.1900	91.1422	2.094	86.9243	95.3601	-5.9522
40	98.0200	91.1422	2.094	86.9243	95.3601	6.8778
41	81.9000	91.1422	2.094	86.9243	95.3601	-9.2422
42	81.5500	91.1422	2.094	86.9243	95.3601	-9.5922
43	85.8300	73.0979	3.171	66.7111	79.4846	12.7321
44	70.5600	73.0979	3.171	66.7111	79.4846	-2.5379
45	77.1700	73.0979	3.171	66.7111	79.4846	4.0721
46	76.2200	73.0979	3.171	66.7111	79.4846	3.1221
47	68.5500	73.0979	3.171	66.7111	79.4846	-4.5479
48	74.1600	73.0979	3.171	66.7111	79.4846	1.0621

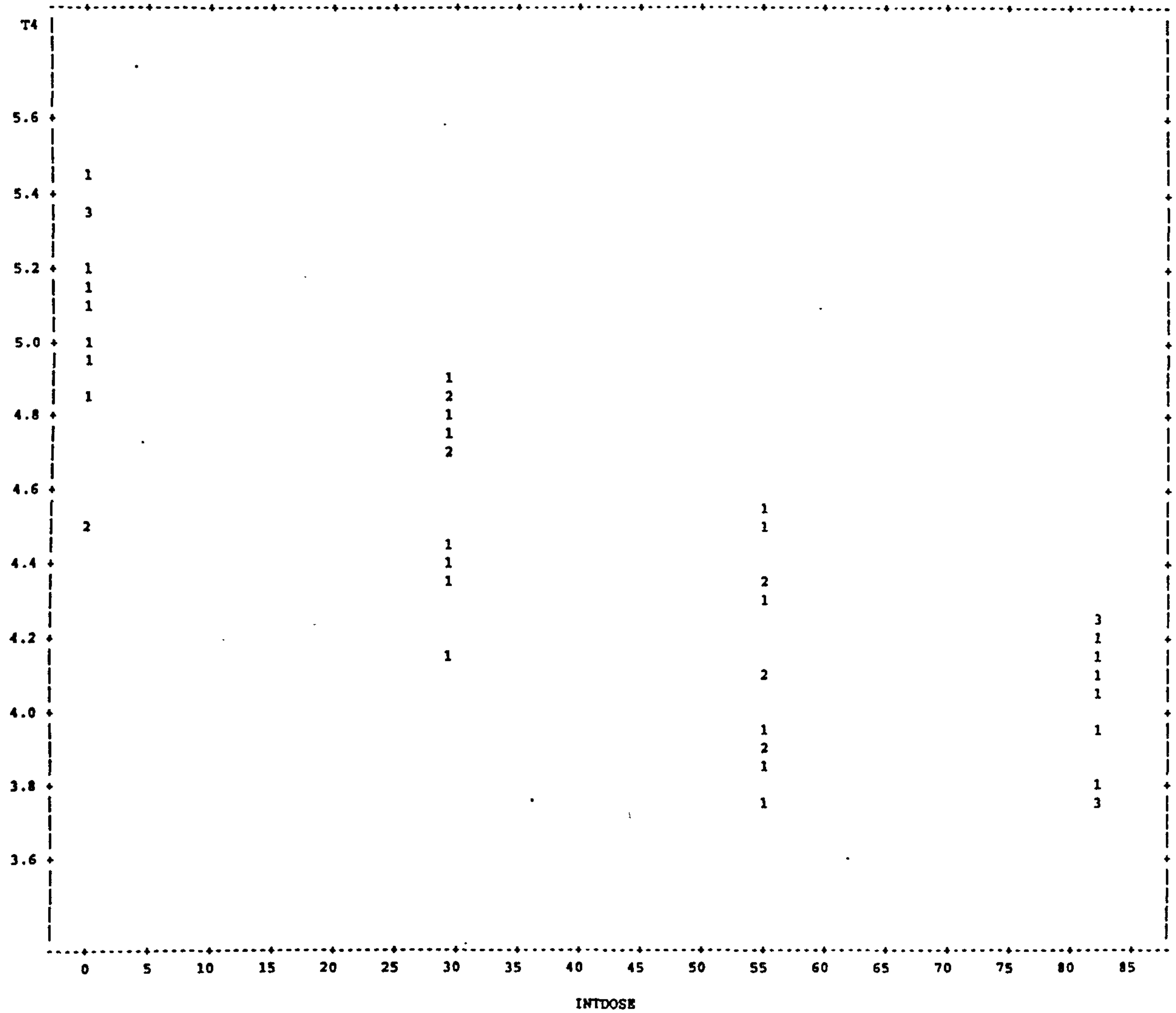
Sum of Residuals 0
Sum of Squared Residuals 7820.8130
Predicted Resid SS (Press) 8377.3462

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	10.9200	11.6967	1.015	9.6532	13.7402	-0.7767
2	10.7100	11.6967	1.015	9.6532	13.7402	-0.9867
3	12.0100	11.6967	1.015	9.6532	13.7402	0.3133
4	11.4000	11.6967	1.015	9.6532	13.7402	-0.2967
5	10.9600	11.6967	1.015	9.6532	13.7402	-0.7367
6	11.4900	11.6967	1.015	9.6532	13.7402	-0.2067
7	11.9600	15.5477	0.651	14.2373	16.8582	-3.5877
8	11.7100	15.5477	0.651	14.2373	16.8582	-3.8377
9	14.7400	15.5477	0.651	14.2373	16.8582	-0.8077
10	13.4200	15.5477	0.651	14.2373	16.8582	-2.1277
11	13.6800	15.5477	0.651	14.2373	16.8582	-1.8677
12	12.8100	15.5477	0.651	14.2373	16.8582	-2.7377
13	15.4500	19.0004	0.654	17.6828	20.3180	-3.5504
14	12.5400	19.0004	0.654	17.6828	20.3180	-6.4604
15	16.4200	19.0004	0.654	17.6828	20.3180	-2.5804
16	15.9300	19.0004	0.654	17.6828	20.3180	-3.0704
17	16.4300	19.0004	0.654	17.6828	20.3180	-2.5704
18	15.3700	19.0004	0.654	17.6828	20.3180	-3.6304
19	17.1700	22.5858	0.991	20.5907	24.5809	-5.4158
20	17.2100	22.5858	0.991	20.5907	24.5809	-5.3758
21	17.3300	22.5858	0.991	20.5907	24.5809	-5.2558
22	17.5500	22.5858	0.991	20.5907	24.5809	-5.0358
23	16.7700	22.5858	0.991	20.5907	24.5809	-5.8158

14	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
15	0	0.9252	0.082	0.7598	1.0905	-0.9252
16	0	0.9252	0.082	0.7598	1.0905	-0.9252
17	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
18	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
19	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
20	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
21	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
22	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
23	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
24	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
25	0	0.2634	0.127	0.00700	0.5199	-0.2634
26	1.0000	0.2634	0.127	0.00700	0.5199	0.7366
27	0	0.2634	0.127	0.00700	0.5199	-0.2634
28	0	0.2634	0.127	0.00700	0.5199	-0.2634
29	0	0.2634	0.127	0.00700	0.5199	-0.2634
30	1.0000	0.2634	0.127	0.00700	0.5199	0.7366
31	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
32	0	0.6124	0.082	0.4479	0.7768	-0.6124
33	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
34	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
35	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
36	1.0000	0.6124	0.082	0.4479	0.7768	0.3876
37	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
38	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
39	0	0.9252	0.082	0.7598	1.0905	-0.9252
40	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
41	2.0000	0.9252	0.082	0.7598	1.0905	1.0748
42	1.0000	0.9252	0.082	0.7598	1.0905	0.0748
43	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
44	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
45	2.0000	1.2500	0.124	0.9997	1.5004	0.7500
46	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
47	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500
48	1.0000	1.2500	0.124	0.9997	1.5004	-0.2500

Sum of Residuals	0
Sum of Squared Residuals	12.0196
Predicted Resid SS (Press)	13.0729

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.4631	0.132	0.1966	0.7297	0.5369
2	1.0000	0.4631	0.132	0.1966	0.7297	0.5369
3	1.0000	0.4631	0.132	0.1966	0.7297	0.5369


T4
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
INTDOSE

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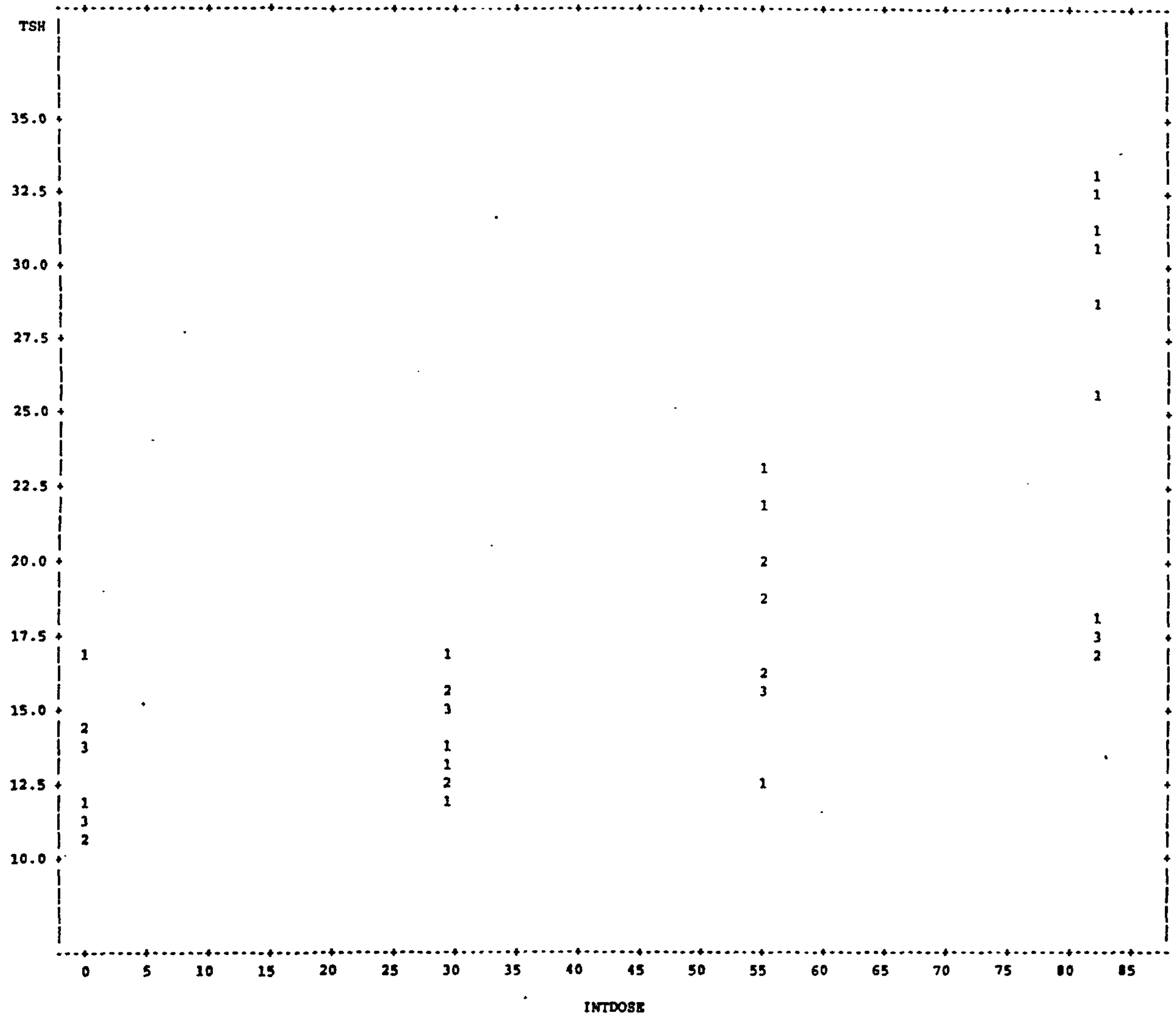


Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	835.91606	835.91606	53.157	0.0001
Error	45	707.63992	15.72533		
C Total	46	1543.55598			

Root MSE	3.96552	R-square	0.5416
Dep Mean	17.24298	Adj R-sq	0.5314
C.V.	22.99787		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	13.601987	0.76417937	17.799	0.0001
DCORR	1	3.485267	0.47802945	7.291	0.0001

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6.40318	6.40318	23.967	0.0001
Error	45	12.02235	0.26716		
C Total	46	18.42553			

Root MSE	0.51688	R-square	0.3475
Dep Mean	0.76596	Adj R-sq	0.3330
C.V.	67.48135		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.447291	0.09960561	4.491	0.0001
DCORR	1	0.305037	0.06230790	4.896	0.0001

21	3.8000	3.9079	0.099	3.7089	4.1069	-0.1079
22	3.7500	3.9079	0.099	3.7089	4.1069	-0.1579
23	4.1100	3.9079	0.099	3.7089	4.1069	0.2021
24	3.7600	3.9079	0.099	3.7089	4.1069	-0.1479
25	5.1900	4.7511	0.069	4.6127	4.8894	0.4389
26	5.1200	4.7511	0.069	4.6127	4.8894	0.3689
27	5.1600	4.7511	0.069	4.6127	4.8894	0.4089
28	4.9800	4.7511	0.069	4.6127	4.8894	0.2289
29	5.4400	4.7511	0.069	4.6127	4.8894	0.6889
30	4.8500	4.7511	0.069	4.6127	4.8894	0.0989
31	4.8600	4.7230	0.066	4.5901	4.8558	0.1370
32	4.7900	4.7230	0.066	4.5901	4.8558	0.0670
33	4.8300	4.7230	0.066	4.5901	4.8558	0.1070
34	4.7300	4.7230	0.066	4.5901	4.8558	0.00704
35	4.7000	4.7230	0.066	4.5901	4.8558	-0.0230
36	4.9100	4.7230	0.066	4.5901	4.8558	0.1870
37	4.3500	4.4700	0.052	4.3652	4.5748	-0.1200
38	4.3400	4.4700	0.052	4.3652	4.5748	-0.1300
39	3.9200	4.4700	0.052	4.3652	4.5748	-0.5500
40	4.5300	4.4700	0.052	4.3652	4.5748	0.0600
41	4.2900	4.4700	0.052	4.3652	4.5748	-0.1800
42	4.4900	4.4700	0.052	4.3652	4.5748	0.0200
43	4.0600	3.9079	0.099	3.7089	4.1069	0.1521
44	4.2600	3.9079	0.099	3.7089	4.1069	0.3521
45	4.1600	3.9079	0.099	3.7089	4.1069	0.2521
46	4.2500	3.9079	0.099	3.7089	4.1069	0.3421
47	4.2300	3.9079	0.099	3.7089	4.1069	0.3221
48	4.1800	3.9079	0.099	3.7089	4.1069	0.2721

Sum of Residuals	0
Sum of Squared Residuals	5.7157
Predicted Resid SS (Press)	6.1478

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	124.3	114.9	3.354	108.1	121.6	9.4656
2	138.4	114.9	3.354	108.1	121.6	23.4956
3	127.9	114.9	3.354	108.1	121.6	13.0256
4	133.1	114.9	3.354	108.1	121.6	18.2756
5	134.2	114.9	3.354	108.1	121.6	19.3056
6	113.2	114.9	3.354	108.1	121.6	-1.6944
7	93.9100	113.4	3.220	107.0	119.9	-19.5302
8	75.7000	113.4	3.220	107.0	119.9	-37.7402
9	84.1700	113.4	3.220	107.0	119.9	-29.2702
10	85.0400	113.4	3.220	107.0	119.9	-28.4002
11	86.6600	113.4	3.220	107.0	119.9	-26.7802

3	12.0100	13.6020	0.764	12.0629	15.1411	-1.5920
4	11.4000	13.6020	0.764	12.0629	15.1411	-2.2020
5	10.9600	13.6020	0.764	12.0629	15.1411	-2.6420
6	11.4900	13.6020	0.764	12.0629	15.1411	-2.1120
7	11.9600	13.9505	0.734	12.4725	15.4285	-1.9905
8	11.7100	13.9505	0.734	12.4725	15.4285	-2.2405
9	14.7400	13.9505	0.734	12.4725	15.4285	0.7895
10	13.4200	13.9505	0.734	12.4725	15.4285	-0.5305
11	13.6800	13.9505	0.734	12.4725	15.4285	-0.2705
12	12.8100	13.9505	0.734	12.4725	15.4285	-1.1405
13	15.4500	17.0873	0.579	15.9214	18.2531	-1.6373
14	12.5400	17.0873	0.579	15.9214	18.2531	-4.5473
15	16.4200	17.0873	0.579	15.9214	18.2531	-0.6673
16	15.9300	17.0873	0.579	15.9214	18.2531	-1.1573
17	16.4300	17.0873	0.579	15.9214	18.2531	-0.6573
18	15.3700	17.0873	0.579	15.9214	18.2531	-1.7173
19	17.1700	24.0578	1.099	21.8439	26.2717	-6.8878
20	17.2100	24.0578	1.099	21.8439	26.2717	-6.8478
21	17.3300	24.0578	1.099	21.8439	26.2717	-6.7278
22	17.5500	24.0578	1.099	21.8439	26.2717	-6.5078
23	16.7700	24.0578	1.099	21.8439	26.2717	-7.2878
24	18.2900	24.0578	1.099	21.8439	26.2717	-5.7678
25	14.2400	13.6020	0.764	12.0629	15.1411	0.6380
26	13.7600	13.6020	0.764	12.0629	15.1411	0.1580
27	14.6800	13.6020	0.764	12.0629	15.1411	1.0780
28	13.8800	13.6020	0.764	12.0629	15.1411	0.2780
29	16.6900	13.6020	0.764	12.0629	15.1411	3.0880
30	13.5900	13.6020	0.764	12.0629	15.1411	-0.0120
31	14.8700	13.9505	0.734	12.4725	15.4285	0.9195
32	15.0300	13.9505	0.734	12.4725	15.4285	1.0795
33	12.3200	13.9505	0.734	12.4725	15.4285	-1.6305
34	15.8500	13.9505	0.734	12.4725	15.4285	1.8995
35	16.7000	13.9505	0.734	12.4725	15.4285	2.7495
36	15.3500	13.9505	0.734	12.4725	15.4285	1.3995
37	22.8800	17.0873	0.579	15.9214	18.2531	5.7927
38	21.7400	17.0873	0.579	15.9214	18.2531	4.6527
39	18.7900	17.0873	0.579	15.9214	18.2531	1.7027
40	18.4500	17.0873	0.579	15.9214	18.2531	1.3627
41	19.9200	17.0873	0.579	15.9214	18.2531	2.8327
42	19.7200	17.0873	0.579	15.9214	18.2531	2.6327
43	32.6600	24.0578	1.099	21.8439	26.2717	8.6022
44	28.7600	24.0578	1.099	21.8439	26.2717	4.7022
45	30.4100	24.0578	1.099	21.8439	26.2717	6.3522
46	31.1800	24.0578	1.099	21.8439	26.2717	7.1222
47	25.5700	24.0578	1.099	21.8439	26.2717	1.5122
48	32.8200	24.0578	1.099	21.8439	26.2717	8.7622

Sum of Residuals 0
Sum of Squared Residuals 707.6399

43	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
44	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
45	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
46	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
47	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
48	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624

Sum of Residuals	0
Sum of Squared Residuals	12.0223
Predicted Resid SS (Press)	13.0737

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
2	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
3	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
4	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
5	0	0.6026	0.102	0.3969	0.8084	-0.6026
6	0	0.6026	0.102	0.3969	0.8084	-0.6026
7	0	0.6284	0.098	0.4308	0.8260	-0.6284
8	.	0.6284	0.098	0.4308	0.8260	.
9	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
10	0	0.6284	0.098	0.4308	0.8260	-0.6284
11	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
12	0	0.6284	0.098	0.4308	0.8260	-0.6284
13	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
14	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
15	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
16	0	0.8608	0.077	0.7050	1.0167	-0.8608
17	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
18	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
19	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
20	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
21	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
22	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
23	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
24	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
25	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
26	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
27	0	0.6026	0.102	0.3969	0.8084	-0.6026
28	0	0.6026	0.102	0.3969	0.8084	-0.6026
29	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
30	0	0.6026	0.102	0.3969	0.8084	-0.6026
31	0	0.6284	0.098	0.4308	0.8260	-0.6284
32	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
33	0	0.6284	0.098	0.4308	0.8260	-0.6284

1 T4, T3, TSH, and Follicle Hyperplasia and follicular lumen 09:46 Monday, February 8, 1999 1
area data from Caldwell 14-day perchlorate study with PK internal dose approximation from Channel, 1999

OBS	ID	DOSE	SEX	FH	FL	T4	T3	TSH	DCORR	INTDOSE
1	68	0.00	F	1	1	4.49	124.33	10.92	0.0	0.0
2	71	0.00	F	1	1	4.50	138.36	10.71	0.0	0.0
3	86	0.00	F	0	1	5.36	127.89	12.01	0.0	0.0
4	90	0.00	F	0	1	5.36	133.14	11.40	0.0	0.0
5	95	0.00	F	0	0	4.94	134.17	10.96	0.0	0.0
6	100	0.00	F	0	0	5.33	113.17	11.49	0.0	0.0
7	51	1.25	F	0	0	4.41	93.91	11.96	0.1	-17.5
8	55	1.25	F	.	.	4.37	75.70	11.71	0.1	-17.5
9	58	1.25	F	0	1	4.47	84.17	14.74	0.1	-17.5
10	67	1.25	F	1	0	4.37	85.04	13.42	0.1	-17.5
11	73	1.25	F	0	1	4.13	86.66	13.68	0.1	-17.5
12	84	1.25	F	0	0	4.69	82.11	12.81	0.1	-17.5
13	60	12.50	F	1	1	3.75	94.00	15.45	1.0	-9.0
14	66	12.50	F	1	1	3.84	79.42	12.54	1.0	-9.0
15	70	12.50	F	0	1	4.11	76.16	16.42	1.0	-9.0
16	77	12.50	F	0	0	3.97	77.21	15.93	1.0	-9.0
17	92	12.50	F	1	1	4.11	78.67	16.43	1.0	-9.0
18	99	12.50	F	1	1	3.92	78.61	15.37	1.0	-9.0
19	59	25.00	F	2	1	3.75	73.91	17.17	3.0	3.0
20	64	25.00	F	1	1	3.96	85.00	17.21	3.0	3.0
21	76	25.00	F	1	2	3.80	73.47	17.33	3.0	3.0
22	80	25.00	F	1	1	3.75	74.16	17.55	3.0	3.0
23	87	25.00	F	2	2	4.11	88.61	16.77	3.0	3.0
24	94	25.00	F	1	1	3.76	80.65	18.29	3.0	3.0
25	11	0.00	M	0	1	5.19	139.85	14.24	0.0	0.0
26	15	0.00	M	1	1	5.12	141.54	13.76	0.0	0.0
27	24	0.00	M	0	0	5.16	136.13	14.68	0.0	0.0
28	39	0.00	M	0	0	4.98	110.68	13.88	0.0	0.0
29	44	0.00	M	0	1	5.44	129.32	16.69	0.0	0.0
30	47	0.00	M	1	0	4.85	139.67	13.59	0.0	0.0
31	1	1.25	M	1	0	4.86	132.68	14.87	0.1	-17.5
32	12	1.25	M	0	1	4.79	127.29	15.03	0.1	-17.5
33	14	1.25	M	1	0	4.83	137.89	12.32	0.1	-17.5
34	19	1.25	M	1	1	4.73	126.69	15.85	0.1	-17.5
35	25	1.25	M	1	1	4.70	106.41	16.70	0.1	-17.5
36	27	1.25	M	1	1	4.91	113.15	15.35	0.1	-17.5
37	17	12.50	M	1	2	4.35	99.33	22.88	1.0	-9.0
38	20	12.50	M	1	1	4.34	96.77	21.74	1.0	-9.0
39	26	12.50	M	0	1	3.92	85.19	18.79	1.0	-9.0
40	38	12.50	M	1	0	4.53	98.02	18.45	1.0	-9.0
41	41	12.50	M	2	2	4.29	81.90	19.92	1.0	-9.0
42	45	12.50	M	1	1	4.49	81.55	19.72	1.0	-9.0
43	4	25.00	M	1	2	4.06	85.83	32.66	3.0	3.0
44	7	25.00	M	2	1	4.26	70.56	28.76	3.0	3.0

TSH	0.74258	0.73973	0.36989	-0.38004	-0.50939	1.00000	0.46247	0.48478
	0.0001	0.0001	0.0097	0.0077	0.0002	0.0	0.0011	0.0006
	48	48	48	48	48	48	47	47
FH	0.59512	0.58951	0.23805	-0.46378	-0.34372	0.46247	1.00000	0.48237
	0.0001	0.0001	0.1071	0.0010	0.0180	0.0011	0.0	0.0006
	47	47	47	47	47	47	47	47
FL	0.53089	0.51594	0.29103	-0.38990	-0.34003	0.48478	0.48237	1.00000
	0.0001	0.0002	0.0472	0.0067	0.0194	0.0006	0.0006	0.0
	47	47	47	47	47	47	47	47

Correlation Analysis

Spearman Correlation Coefficients / Prob > |R| under Ho: Rho=0 / Number of Observations

	DOSE	DCORR	INTDOSE	T4	T3	TSH	FH	FL
DOSE	1.00000	1.00000	0.40000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0	0.0001	0.0048	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
DCORR	1.00000	1.00000	0.40000	-0.83826	-0.81379	0.81243	0.59016	0.49760
	0.0001	0.0	0.0048	0.0001	0.0001	0.0001	0.0001	0.0004
	48	48	48	48	48	48	47	47
INTDOSE	0.40000	0.40000	1.00000	-0.25363	-0.25288	0.39680	0.31695	0.35173
	0.0048	0.0048	0.0	0.0820	0.0829	0.0052	0.0300	0.0153
	48	48	48	48	48	48	47	47
T4	-0.83826	-0.83826	-0.25363	1.00000	0.77215	-0.50928	-0.43423	-0.41697
	0.0001	0.0001	0.0820	0.0	0.0001	0.0002	0.0023	0.0035
	48	48	48	48	48	48	47	47
T3	-0.81379	-0.81379	-0.25288	0.77215	1.00000	-0.56564	-0.35229	-0.33194
	0.0001	0.0001	0.0829	0.0001	0.0	0.0001	0.0152	0.0226
	48	48	48	48	48	48	47	47
TSH	0.81243	0.81243	0.39680	-0.50928	-0.56564	1.00000	0.50107	0.54035
	0.0001	0.0001	0.0052	0.0002	0.0001	0.0	0.0003	0.0001
	48	48	48	48	48	48	47	47
FH	0.59016	0.59016	0.31695	-0.43423	-0.35229	0.50107	1.00000	0.45934
	0.0001	0.0001	0.0300	0.0023	0.0152	0.0003	0.0	0.0012
	47	47	47	47	47	47	47	47
FL	0.49760	0.49760	0.35173	-0.41697	-0.33194	0.54035	0.45934	1.00000
	0.0004	0.0004	0.0153	0.0035	0.0226	0.0001	0.0012	0.0

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	191.23320	191.23320	6.363	0.0153
Error	45	1352.32278	30.05162		
C Total	46	1543.55598			

Root MSE	5.48194	R-square	0.1239
Dep Mean	17.24298	Adj R-sq	0.1044
C.V.	31.79228		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	18.673342	0.98025930	19.049	0.0001
INTDOSE	1	0.254167	0.10075600	2.523	0.0153

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	1.04414	1.04414	2.703	0.1071
Error	45	17.38140	0.38625		
C Total	46	18.42553			

Root MSE	0.62149	R-square	0.0567
Dep Mean	0.76596	Adj R-sq	0.0357
C.V.	81.13933		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.871650	0.11113302	7.843	0.0001
INTDOSE	1	0.018781	0.01142281	1.644	0.1071

20	3.9600	4.4185	0.107	4.2031	4.6340	-0.4585
21	3.8000	4.4185	0.107	4.2031	4.6340	-0.6185
22	3.7500	4.4185	0.107	4.2031	4.6340	-0.6685
23	4.1100	4.4185	0.107	4.2031	4.6340	-0.3085
24	3.7600	4.4185	0.107	4.2031	4.6340	-0.6585
25	5.1900	4.4321	0.089	4.2533	4.6109	0.7579
26	5.1200	4.4321	0.089	4.2533	4.6109	0.6879
27	5.1600	4.4321	0.089	4.2533	4.6109	0.7279
28	4.9800	4.4321	0.089	4.2533	4.6109	0.5479
29	5.4400	4.4321	0.089	4.2533	4.6109	1.0079
30	4.8500	4.4321	0.089	4.2533	4.6109	0.4179
31	4.8600	4.5110	0.130	4.2485	4.7734	0.3490
32	4.7900	4.5110	0.130	4.2485	4.7734	0.2790
33	4.8300	4.5110	0.130	4.2485	4.7734	0.3190
34	4.7300	4.5110	0.130	4.2485	4.7734	0.2190
35	4.7000	4.5110	0.130	4.2485	4.7734	0.1890
36	4.9100	4.5110	0.130	4.2485	4.7734	0.3990
37	4.3500	4.4727	0.079	4.3142	4.6311	-0.1227
38	4.3400	4.4727	0.079	4.3142	4.6311	-0.1327
39	3.9200	4.4727	0.079	4.3142	4.6311	-0.5527
40	4.5300	4.4727	0.079	4.3142	4.6311	0.0573
41	4.2900	4.4727	0.079	4.3142	4.6311	-0.1827
42	4.4900	4.4727	0.079	4.3142	4.6311	0.0173
43	4.0600	4.4185	0.107	4.2031	4.6340	-0.3585
44	4.2600	4.4185	0.107	4.2031	4.6340	-0.1585
45	4.1600	4.4185	0.107	4.2031	4.6340	-0.2585
46	4.2500	4.4185	0.107	4.2031	4.6340	-0.1685
47	4.2300	4.4185	0.107	4.2031	4.6340	-0.1885
48	4.1800	4.4185	0.107	4.2031	4.6340	-0.2385

Sum of Residuals	0
Sum of Squared Residuals	11.0917
Predicted Resid SS (Press)	11.9466

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	124.3	98.8647	4.418	89.9666	107.8	25.4653
2	138.4	98.8647	4.418	89.9666	107.8	39.4953
3	127.9	98.8647	4.418	89.9666	107.8	29.0253
4	133.1	98.8647	4.418	89.9666	107.8	34.2753
5	134.2	98.8647	4.418	89.9666	107.8	35.3053
6	113.2	98.8647	4.418	89.9666	107.8	14.3053
7	93.9100	102.4	6.485	89.2926	115.4	-8.4434
8	75.7000	102.4	6.485	89.2926	115.4	-26.6534
9	84.1700	102.4	6.485	89.2926	115.4	-18.1834
10	85.0400	102.4	6.485	89.2926	115.4	-17.3134

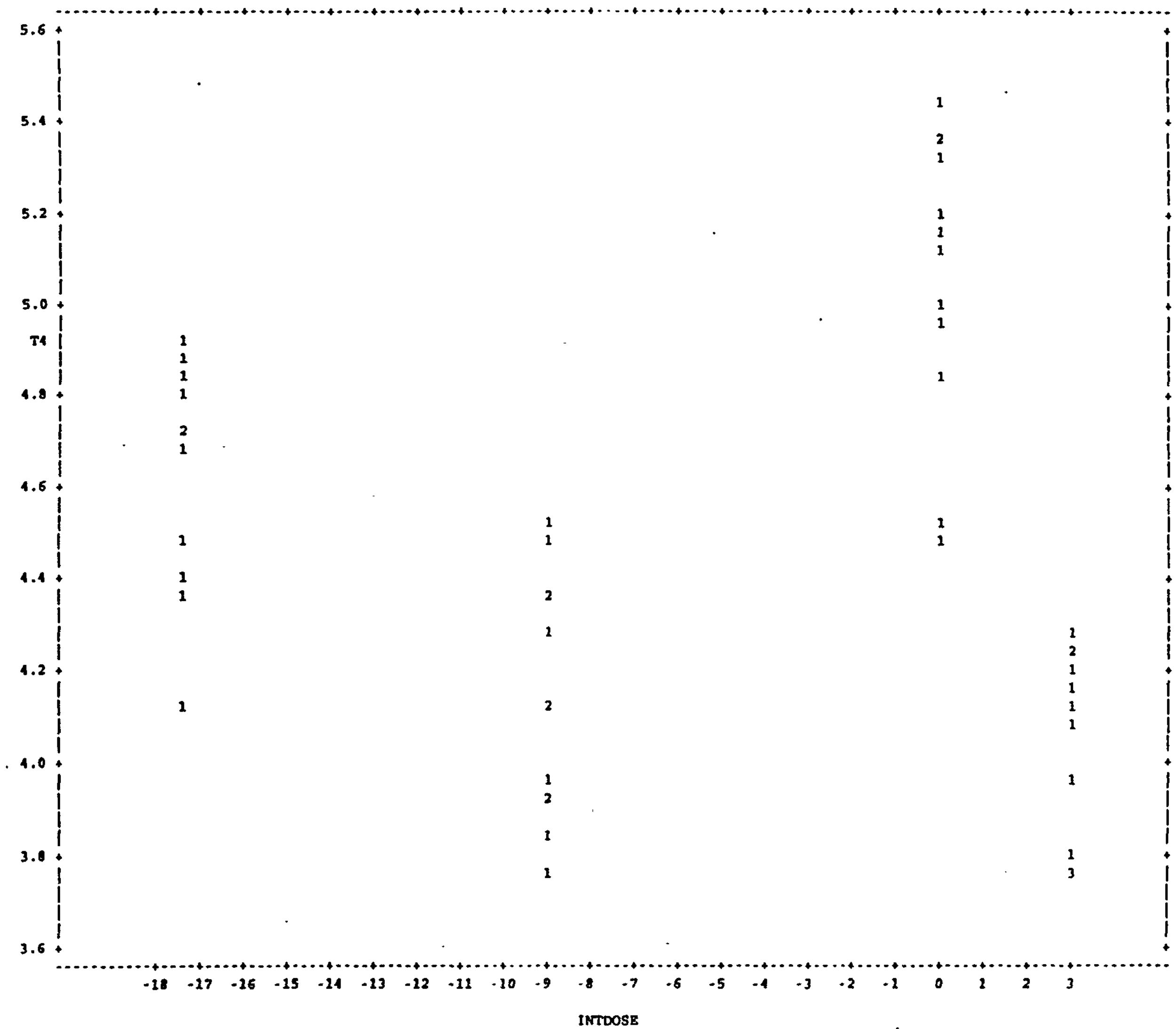
2	10.7100	18.6733	0.980	16.6990	20.6477	-7.9633
3	12.0100	18.6733	0.980	16.6990	20.6477	-6.6633
4	11.4000	18.6733	0.980	16.6990	20.6477	-7.2733
5	10.9600	18.6733	0.980	16.6990	20.6477	-7.7133
6	11.4900	18.6733	0.980	16.6990	20.6477	-7.1833
7	11.9600	14.2254	1.439	11.3274	17.1234	-2.2654
8	11.7100	14.2254	1.439	11.3274	17.1234	-2.5154
9	14.7400	14.2254	1.439	11.3274	17.1234	0.5146
10	13.4200	14.2254	1.439	11.3274	17.1234	-0.8054
11	13.6800	14.2254	1.439	11.3274	17.1234	-0.5454
12	12.8100	14.2254	1.439	11.3274	17.1234	-1.4154
13	15.4500	16.3858	0.869	14.6359	18.1357	-0.9358
14	12.5400	16.3858	0.869	14.6359	18.1357	-3.8458
15	16.4200	16.3858	0.869	14.6359	18.1357	0.0342
16	15.9300	16.3858	0.869	14.6359	18.1357	-0.4558
17	16.4300	16.3858	0.869	14.6359	18.1357	0.0442
18	15.3700	16.3858	0.869	14.6359	18.1357	-1.0158
19	17.1700	19.4358	1.181	17.0569	21.8148	-2.2658
20	17.2100	19.4358	1.181	17.0569	21.8148	-2.2258
21	17.3300	19.4358	1.181	17.0569	21.8148	-2.1058
22	17.5500	19.4358	1.181	17.0569	21.8148	-1.8858
23	16.7700	19.4358	1.181	17.0569	21.8148	-2.6658
24	18.2900	19.4358	1.181	17.0569	21.8148	-1.1458
25	14.2400	18.6733	0.980	16.6990	20.6477	-4.4333
26	13.7600	18.6733	0.980	16.6990	20.6477	-4.9133
27	14.6800	18.6733	0.980	16.6990	20.6477	-3.9933
28	13.8800	18.6733	0.980	16.6990	20.6477	-4.7933
29	16.6900	18.6733	0.980	16.6990	20.6477	-1.9833
30	13.5900	18.6733	0.980	16.6990	20.6477	-5.0833
31	14.8700	14.2254	1.439	11.3274	17.1234	0.6446
32	15.0300	14.2254	1.439	11.3274	17.1234	0.8046
33	12.3200	14.2254	1.439	11.3274	17.1234	-1.9054
34	15.8500	14.2254	1.439	11.3274	17.1234	1.6246
35	16.7000	14.2254	1.439	11.3274	17.1234	2.4746
36	15.3500	14.2254	1.439	11.3274	17.1234	1.1246
37	22.8800	16.3858	0.869	14.6359	18.1357	6.4942
38	21.7400	16.3858	0.869	14.6359	18.1357	5.3542
39	18.7900	16.3858	0.869	14.6359	18.1357	2.4042
40	18.4500	16.3858	0.869	14.6359	18.1357	2.0642
41	19.9200	16.3858	0.869	14.6359	18.1357	3.5342
42	19.7200	16.3858	0.869	14.6359	18.1357	3.3342
43	32.6600	19.4358	1.181	17.0569	21.8148	13.2242
44	28.7600	19.4358	1.181	17.0569	21.8148	9.3242
45	30.4100	19.4358	1.181	17.0569	21.8148	10.9742
46	31.1800	19.4358	1.181	17.0569	21.8148	11.7442
47	25.5700	19.4358	1.181	17.0569	21.8148	6.1342
48	32.8200	19.4358	1.181	17.0569	21.8148	13.3842

Sum of Residuals 0

42	1.0000	0.7026	0.098	0.5042	0.9010	0.2974
43	1.0000	0.9280	0.134	0.6583	1.1977	0.0720
44	2.0000	0.9280	0.134	0.6583	1.1977	1.0720
45	2.0000	0.9280	0.134	0.6583	1.1977	1.0720
46	1.0000	0.9280	0.134	0.6583	1.1977	0.0720
47	1.0000	0.9280	0.134	0.6583	1.1977	0.0720
48	1.0000	0.9280	0.134	0.6583	1.1977	0.0720

Sum of Residuals	0
Sum of Squared Residuals	17.3814
Predicted Resid SS (Press)	18.8772

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
2	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
3	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
4	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
5	0	0.9973	0.106	0.7841	1.2105	-0.9973
6	0	0.9973	0.106	0.7841	1.2105	-0.9973
7	0	0.6087	0.155	0.2957	0.9217	-0.6087
8	.	0.6087	0.155	0.2957	0.9217	.
9	1.0000	0.6087	0.155	0.2957	0.9217	0.3913
10	0	0.6087	0.155	0.2957	0.9217	-0.6087
11	1.0000	0.6087	0.155	0.2957	0.9217	0.3913
12	0	0.6087	0.155	0.2957	0.9217	-0.6087
13	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
14	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
15	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
16	0	0.7975	0.094	0.6085	0.9864	-0.7975
17	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
18	1.0000	0.7975	0.094	0.6085	0.9864	0.2025
19	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
20	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
21	2.0000	1.0639	0.128	0.8070	1.3209	0.9361
22	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
23	2.0000	1.0639	0.128	0.8070	1.3209	0.9361
24	1.0000	1.0639	0.128	0.8070	1.3209	-0.0639
25	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
26	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
27	0	0.9973	0.106	0.7841	1.2105	-0.9973
28	0	0.9973	0.106	0.7841	1.2105	-0.9973
29	1.0000	0.9973	0.106	0.7841	1.2105	0.00269
30	0	0.9973	0.106	0.7841	1.2105	-0.9973
31	0	0.6087	0.155	0.2957	0.9217	-0.6087
32	1.0000	0.6087	0.155	0.2957	0.9217	0.3913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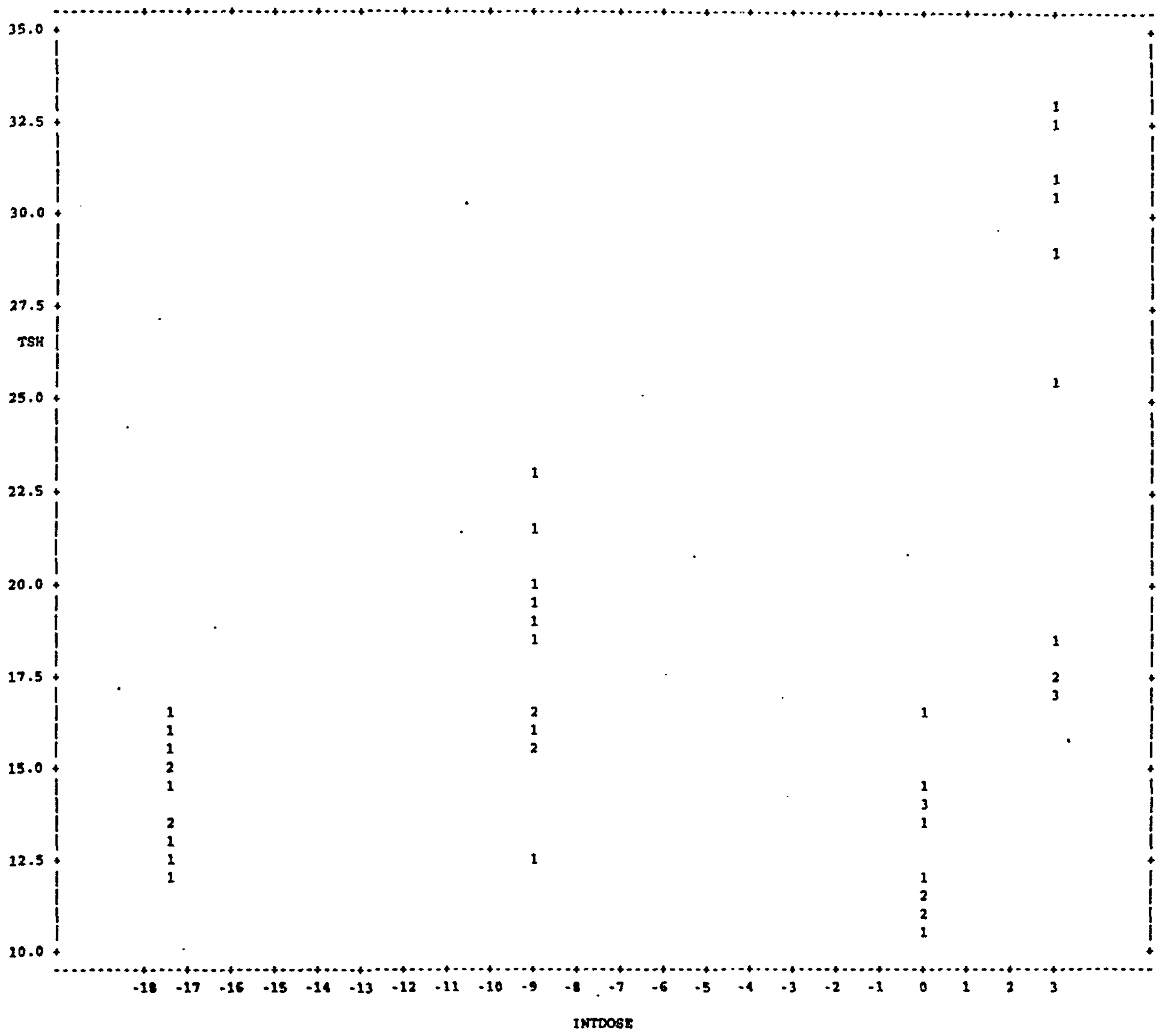
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INTDOSE

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	835.91606	835.91606	53.157	0.0001
Error	45	707.63992	15.72533		
C Total	46	1543.55598			

Root MSE	3.96552	R-square	0.5416
Dep Mean	17.24298	Adj R-sq	0.5314
C.V.	22.99787		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	13.601987	0.76417937	17.799	0.0001
DCORR	1	3.485267	0.47802945	7.291	0.0001

Dependent Variable: FH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6.40318	6.40318	23.967	0.0001
Error	45	12.02235	0.26716		
C Total	46	18.42553			

Root MSE	0.51688	R-square	0.3475
Dep Mean	0.76596	Adj R-sq	0.3330
C.V.	67.48135		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	0.447291	0.09960561	4.491	0.0001
DCORR	1	0.305037	0.06230790	4.896	0.0001

21	3.8000	3.9079	0.099	3.7089	4.1069	-0.1079
22	3.7500	3.9079	0.099	3.7089	4.1069	-0.1579
23	4.1100	3.9079	0.099	3.7089	4.1069	0.2021
24	3.7600	3.9079	0.099	3.7089	4.1069	-0.1479
25	5.1900	4.7511	0.069	4.6127	4.8894	0.4389
26	5.1200	4.7511	0.069	4.6127	4.8894	0.3689
27	5.1600	4.7511	0.069	4.6127	4.8894	0.4089
28	4.9800	4.7511	0.069	4.6127	4.8894	0.2289
29	5.4400	4.7511	0.069	4.6127	4.8894	0.6889
30	4.8500	4.7511	0.069	4.6127	4.8894	0.0989
31	4.8600	4.7230	0.066	4.5901	4.8558	0.1370
32	4.7900	4.7230	0.066	4.5901	4.8558	0.0670
33	4.8300	4.7230	0.066	4.5901	4.8558	0.1070
34	4.7300	4.7230	0.066	4.5901	4.8558	0.00704
35	4.7000	4.7230	0.066	4.5901	4.8558	-0.0230
36	4.9100	4.7230	0.066	4.5901	4.8558	0.1870
37	4.3500	4.4700	0.052	4.3652	4.5748	-0.1200
38	4.3400	4.4700	0.052	4.3652	4.5748	-0.1300
39	3.9200	4.4700	0.052	4.3652	4.5748	-0.5500
40	4.5300	4.4700	0.052	4.3652	4.5748	0.0600
41	4.2900	4.4700	0.052	4.3652	4.5748	-0.1800
42	4.4900	4.4700	0.052	4.3652	4.5748	0.0200
43	4.0600	3.9079	0.099	3.7089	4.1069	0.1521
44	4.2600	3.9079	0.099	3.7089	4.1069	0.3521
45	4.1600	3.9079	0.099	3.7089	4.1069	0.2521
46	4.2500	3.9079	0.099	3.7089	4.1069	0.3421
47	4.2300	3.9079	0.099	3.7089	4.1069	0.3221
48	4.1800	3.9079	0.099	3.7089	4.1069	0.2721

Sum of Residuals	0
Sum of Squared Residuals	5.7157
Predicted Resid SS (Press)	6.1478

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	124.3	114.9	3.354	108.1	121.6	9.4656
2	138.4	114.9	3.354	108.1	121.6	23.4956
3	127.9	114.9	3.354	108.1	121.6	13.0256
4	133.1	114.9	3.354	108.1	121.6	18.2756
5	134.2	114.9	3.354	108.1	121.6	19.3056
6	113.2	114.9	3.354	108.1	121.6	-1.6944
7	93.9100	113.4	3.220	107.0	119.9	-19.5302
8	75.7000	113.4	3.220	107.0	119.9	-37.7402
9	84.1700	113.4	3.220	107.0	119.9	-29.2702
10	85.0400	113.4	3.220	107.0	119.9	-28.4002
11	86.6600	113.4	3.220	107.0	119.9	-26.7802

3	12.0100	13.6020	0.764	12.0629	15.1411	-1.5920
4	11.4000	13.6020	0.764	12.0629	15.1411	-2.2020
5	10.9600	13.6020	0.764	12.0629	15.1411	-2.6420
6	11.4900	13.6020	0.764	12.0629	15.1411	-2.1120
7	11.9600	13.9505	0.734	12.4725	15.4285	-1.9905
8	11.7100	13.9505	0.734	12.4725	15.4285	-2.2405
9	14.7400	13.9505	0.734	12.4725	15.4285	0.7895
10	13.4200	13.9505	0.734	12.4725	15.4285	-0.5305
11	13.6800	13.9505	0.734	12.4725	15.4285	-0.2705
12	12.8100	13.9505	0.734	12.4725	15.4285	-1.1405
13	15.4500	17.0873	0.579	15.9214	18.2531	-1.6373
14	12.5400	17.0873	0.579	15.9214	18.2531	-4.5473
15	16.4200	17.0873	0.579	15.9214	18.2531	-0.6673
16	15.9300	17.0873	0.579	15.9214	18.2531	-1.1573
17	16.4300	17.0873	0.579	15.9214	18.2531	-0.6573
18	15.3700	17.0873	0.579	15.9214	18.2531	-1.7173
19	17.1700	24.0578	1.099	21.8439	26.2717	-6.8878
20	17.2100	24.0578	1.099	21.8439	26.2717	-6.8478
21	17.3300	24.0578	1.099	21.8439	26.2717	-6.7278
22	17.5500	24.0578	1.099	21.8439	26.2717	-6.5078
23	16.7700	24.0578	1.099	21.8439	26.2717	-7.2878
24	18.2900	24.0578	1.099	21.8439	26.2717	-5.7678
25	14.2400	13.6020	0.764	12.0629	15.1411	0.6380
26	13.7600	13.6020	0.764	12.0629	15.1411	0.1580
27	14.6800	13.6020	0.764	12.0629	15.1411	1.0780
28	13.8800	13.6020	0.764	12.0629	15.1411	0.2780
29	16.6900	13.6020	0.764	12.0629	15.1411	3.0880
30	13.5900	13.6020	0.764	12.0629	15.1411	-0.0120
31	14.8700	13.9505	0.734	12.4725	15.4285	0.9195
32	15.0300	13.9505	0.734	12.4725	15.4285	1.0795
33	12.3200	13.9505	0.734	12.4725	15.4285	-1.6305
34	15.8500	13.9505	0.734	12.4725	15.4285	1.8995
35	16.7000	13.9505	0.734	12.4725	15.4285	2.7495
36	15.3500	13.9505	0.734	12.4725	15.4285	1.3995
37	22.8800	17.0873	0.579	15.9214	18.2531	5.7927
38	21.7400	17.0873	0.579	15.9214	18.2531	4.6527
39	18.7900	17.0873	0.579	15.9214	18.2531	1.7027
40	18.4500	17.0873	0.579	15.9214	18.2531	1.3627
41	19.9200	17.0873	0.579	15.9214	18.2531	2.8327
42	19.7200	17.0873	0.579	15.9214	18.2531	2.6327
43	32.6600	24.0578	1.099	21.8439	26.2717	8.6022
44	28.7600	24.0578	1.099	21.8439	26.2717	4.7022
45	30.4100	24.0578	1.099	21.8439	26.2717	6.3522
46	31.1800	24.0578	1.099	21.8439	26.2717	7.1222
47	25.5700	24.0578	1.099	21.8439	26.2717	1.5122
48	32.8200	24.0578	1.099	21.8439	26.2717	8.7622

Sum of Residuals 0
Sum of Squared Residuals 707.6399

43	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
44	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
45	2.0000	1.3624	0.143	1.0738	1.6510	0.6376
46	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
47	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624
48	1.0000	1.3624	0.143	1.0738	1.6510	-0.3624

Sum of Residuals	0
Sum of Squared Residuals	12.0223
Predicted Resid SS (Press)	13.0737

Obs	Dep Var FL	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
2	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
3	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
4	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
5	0	0.6026	0.102	0.3969	0.8084	-0.6026
6	0	0.6026	0.102	0.3969	0.8084	-0.6026
7	0	0.6284	0.098	0.4308	0.8260	-0.6284
8	.	0.6284	0.098	0.4308	0.8260	.
9	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
10	0	0.6284	0.098	0.4308	0.8260	-0.6284
11	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
12	0	0.6284	0.098	0.4308	0.8260	-0.6284
13	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
14	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
15	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
16	0	0.8608	0.077	0.7050	1.0167	-0.8608
17	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
18	1.0000	0.8608	0.077	0.7050	1.0167	0.1392
19	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
20	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
21	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
22	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
23	2.0000	1.3772	0.147	1.0812	1.6732	0.6228
24	1.0000	1.3772	0.147	1.0812	1.6732	-0.3772
25	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
26	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
27	0	0.6026	0.102	0.3969	0.8084	-0.6026
28	0	0.6026	0.102	0.3969	0.8084	-0.6026
29	1.0000	0.6026	0.102	0.3969	0.8084	0.3974
30	0	0.6026	0.102	0.3969	0.8084	-0.6026
31	0	0.6284	0.098	0.4308	0.8260	-0.6284
32	1.0000	0.6284	0.098	0.4308	0.8260	0.3716
33	0	0.6284	0.098	0.4308	0.8260	-0.6284

OBS	SEX	TMPT	DOSE	AGE	T4	T3	TSH	CODE	FOLL	INTDOSE	DCORR
1	M	15-18	1	16	5.10	200.14	13.08	n	0	0	0.00
2	M	15-18	1	15	5.64	208.45	13.72	n	0	0	0.00
3	M	15-18	1	17	6.64	186.23	14.20	n	0	0	0.00
4	M	15-18	1	17	5.88	234.21	16.38	n	0	0	0.00
5	M	15-18	1	16	4.84	205.08	13.21	ab	1	0	0.00
6	M	15-18	1	15	5.42	178.53	15.52	n	0	0	0.00
7	M	15-18	1	18	5.29	170.16	17.45	n	0	0	0.00
8	M	15-18	1	17	5.19	187.11	15.30	n	0	0	0.00
9	M	15-18	2	18	5.10	173.23	15.22	n	0	11	0.01
10	M	15-18	2	17	4.23	170.49	12.76	n	0	11	0.01
11	M	15-18	2	17	5.21	154.81	17.63	ab	0	11	0.01
12	M	15-18	2	17	6.03	136.30	18.41	n	0	11	0.01
13	M	15-18	2	18	5.70	183.30	16.49	n	0	11	0.01
14	M	15-18	5	15	5.58	122.39	19.93	n	0	55	1.00
15	M	15-18	5	18	4.84	130.71	20.27	n	0	55	1.00
16	M	15-18	5	15	5.17	139.56	17.39	n	0	55	1.00
17	M	15-18	5	17	4.69	137.44	17.03	ab	0	55	1.00
18	M	15-18	5	18	4.92	133.18	20.87	n	0	55	1.00
19	M	15-18	5	16	6.06	101.61	18.72	n	0	55	1.00
20	M	15-18	5	15	4.81	125.91	21.75	n	0	55	1.00
21	M	15-18	5	17	4.86	109.93	16.11	n	0	55	1.00
22	M	15-18	5	16	5.24	124.57	17.48	n	0	55	1.00
23	M	15-18	5	17	5.74	112.27	18.45	n	0	55	1.00
24	F	15-18	1	16	4.89	133.28	9.89	n	0	0	0.00
25	F	15-18	1	15	4.23	146.98	9.49	n	0	0	0.00
26	F	15-18	1	15	4.45	148.52	9.00	n	0	0	0.00
27	F	15-18	1	16	4.33	113.01	10.51	n	0	0	0.00
28	F	15-18	1	17	4.87	141.79	10.86	n	0	0	0.00
29	F	15-18	1	16	3.47	115.12	11.30	n	0	0	0.00
30	F	15-18	1	17	4.81	125.46	10.27	n	0	0	0.00
31	F	15-18	1	18	4.36	155.28	11.15	n	0	0	0.00
32	F	15-18	1	18	4.75	117.27	10.57	n	0	0	0.00
33	F	15-18	1	18	4.72	133.24	11.36	n	0	0	0.00
34	F	15-18	2	18	4.48	144.32	11.86	n	0	11	0.01
35	F	15-18	2	15	4.39	134.14	11.83	n	0	11	0.01
36	F	15-18	2	15	4.59	134.16	10.33	n	0	11	0.01
37	F	15-18	2	16	4.58	134.89	10.03	ab	0	11	0.01
38	F	15-18	2	16	4.74	142.55	13.47	n	0	11	0.01
39	F	15-18	2	16	4.60	113.00	10.78	n	0	11	0.01
40	F	15-18	2	18	3.24	147.24	13.71	n	0	11	0.01
41	F	15-18	2	17	4.44	128.84	11.46	n	0	11	0.01
42	F	15-18	2	18	3.83	145.91	11.36	n	0	11	0.01
43	F	15-18	2	17	4.34	115.29	12.35	n	0	11	0.01
44	F	15-18	5	16	4.02	139.95	13.07	n	0	55	1.00
45	F	15-18	5	18	3.45	122.78	12.33	n	0	55	1.00

91	F	92-95	1	95	4.75	157.34	15.31	n	0	0	0.00
92	F	92-95	1	95	4.25	154.03	19.14	n	0	0	0.00
93	F	92-95	1	95	4.72	148.72	15.41	n	0	0	0.00
94	F	92-95	2	92	3.61	161.10	17.85	n	0	11	0.01
95	F	92-95	2	92	3.73	133.70	15.76	n	0	11	0.01
96	F	92-95	2	93	3.60	153.42	17.47	n	0	11	0.01
97	F	92-95	2	93	3.36	140.29	14.80	n	0	11	0.01
98	F	92-95	2	93	3.18	122.90	19.76	n	0	11	0.01
99	F	92-95	2	94	3.30	126.23	19.48	n	0	11	0.01
100	F	92-95	2	94	3.10	136.10	15.89	n	0	11	0.01
101	F	92-95	2	95	3.46	158.85	16.64	n	0	11	0.01
102	F	92-95	2	95	4.29	134.94	14.45	n	0	11	0.01
103	F	92-95	2	95	3.58	163.78	15.73	ab	0	11	0.01
104	F	92-95	5	92	3.02	122.86	17.75	n	0	55	1.00
105	F	92-95	5	92	3.10	116.09	18.81	n	0	55	1.00
106	F	92-95	5	93	3.41	116.59	16.26	n	0	55	1.00
107	F	92-95	5	93	2.90	107.73	19.20	n	0	55	1.00
108	F	92-95	5	93	3.78	135.30	18.76	n	0	55	1.00
109	F	92-95	5	94	3.43	117.29	15.69	n	0	55	1.00
110	F	92-95	5	95	3.18	137.19	18.27	n	0	55	1.00
111	F	92-95	5	95	2.79	124.03	14.97	n	0	55	1.00
112	F	92-95	5	95	3.04	125.61	18.93	n	0	55	1.00

1 T4, T3, TSH data from Dev NT, PND 5Perchlorate study including approx of internal dose from Meyer, 1998

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OBS	DOSE	LITTER	TSH	T4	T3	INTDOSE	DCORR
1	1	19508	4.15	3.17	.	0	0.0
2	1	19520	4.23	2.67	88.8	0	0.0
3	1	19523	4.36	3.49	91.1	0	0.0
4	1	19526	4.06	.	97.9	0	0.0
5	1	19530	4.12	3.37	84.0	0	0.0
6	1	19549	4.68	3.67	82.1	0	0.0
7	1	19556	4.19	3.83	84.1	0	0.0
8	1	19558	4.94	3.29	86.0	0	0.0
9	1	19571	4.09	.	86.5	0	0.0
10	1	19580	4.15	3.07	.	0	0.0
11	1	19582	4.30	2.90	.	0	0.0
12	1	19585	4.72	3.52	.	0	0.0
13	1	19608	4.34	3.81	.	0	0.0
14	1	19610	4.94	3.85	.	0	0.0
15	1	19611	5.46	3.54	.	0	0.0
16	1	19614	5.53	3.85	81.5	0	0.0
17	1	19621	4.45	3.11	97.7	0	0.0
18	2	19511	4.60	3.37	94.1	29	0.1
19	2	19514	3.87	3.04	.	29	0.1
20	2	19527	5.01	3.67	77.8	29	0.1
21	2	19533	3.88	2.87	79.5	29	0.1

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1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE from Meyer, 1998

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Correlation Analysis

5 'VAR' Variables: DCORR INTDOSE T4 T3 TSH

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DCORR	66	1.066667	1.215583	70.400000	0	3.000000
INTDOSE	66	42.272727	31.031272	2790.000000	0	82.000000
T4	50	3.158000	0.408841	157.900000	2.280000	3.850000
T3	42	71.304762	21.611717	2994.800000	34.500000	97.900000
TSH	66	4.647576	0.472255	306.740000	3.760000	5.630000

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / Number of Observations

	DCORR	INTDOSE	T4	T3	TSH
DCORR	1.00000	0.91220	-0.67382	-0.94857	0.21926
	0.0	0.0001	0.0001	0.0001	0.0769
	66	66	50	42	66
INTDOSE	0.91220	1.00000	-0.63297	-0.83589	0.24164
	0.0001	0.0	0.0001	0.0001	0.0506
	66	66	50	42	66
T4	-0.67382	-0.63297	1.00000	0.60491	0.01721
	0.0001	0.0001	0.0	0.0001	0.9056
	50	50	50	39	50
T3	-0.94857	-0.83589	0.60491	1.00000	-0.28791
	0.0001	0.0001	0.0001	0.0	0.0645
	42	42	39	42	42
TSH	0.21926	0.24164	0.01721	-0.28791	1.00000
	0.0769	0.0506	0.9056	0.0645	0.0
	66	66	50	42	66

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 6

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Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.56696	0.56696	2.568	0.1175
Error	37	8.16763	0.22075		
C Total	38	8.73459			

Root MSE	0.46984	R-square	0.0649
Dep Mean	4.68718	Adj R-sq	0.0396
C.V.	10.02388		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.506929	0.13531605	33.307	0.0001
INTDOSE	1	0.004035	0.00251806	1.603	0.1175

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 8

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Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	3.1700	3.5209	0.092	3.3341	3.7078	-0.3509
2	2.6700	3.5209	0.092	3.3341	3.7078	-0.8509
3	3.4900	3.5209	0.092	3.3341	3.7078	-0.0309
4	.	3.5209	0.092	3.3341	3.7078	.
5	3.3700	3.5209	0.092	3.3341	3.7078	-0.1509
6	3.6700	3.5209	0.092	3.3341	3.7078	0.1491
7	3.8300	3.5209	0.092	3.3341	3.7078	0.3091
8	3.2900	3.5209	0.092	3.3341	3.7078	-0.2309
9	.	3.5209	0.092	3.3341	3.7078	.
10	3.0700	3.5209	0.092	3.3341	3.7078	-0.4509
11	2.9000	3.5209	0.092	3.3341	3.7078	-0.6209
12	3.5200	3.5209	0.092	3.3341	3.7078	-0.00094
13	3.8100	3.5209	0.092	3.3341	3.7078	0.2891
14	3.8500	3.5209	0.092	3.3341	3.7078	0.3291
15	3.5400	3.5209	0.092	3.3341	3.7078	0.0191
16	3.8500	3.5209	0.092	3.3341	3.7078	0.3291
17	3.1100	3.5209	0.092	3.3341	3.7078	-0.4109
18	3.3700	3.2528	0.058	3.1355	3.3701	0.1172
19	3.0400	3.2528	0.058	3.1355	3.3701	-0.2128
20	3.6700	3.2528	0.058	3.1355	3.3701	0.4172
21	2.8700	3.2528	0.058	3.1355	3.3701	-0.3828

65	2.6400	2.7628	0.082	2.5965	2.9290	-0.1228
66	.	2.7628	0.082	2.5965	2.9290	.

Sum of Residuals	0
Sum of Squared Residuals	3.7944
Predicted Resid SS (Press)	4.2470

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	.	96.9481	3.553	89.7482	104.1	.
2	88.8000	96.9481	3.553	89.7482	104.1	-8.1481
3	91.1000	96.9481	3.553	89.7482	104.1	-5.8481
4	97.9000	96.9481	3.553	89.7482	104.1	0.9519
5	84.0000	96.9481	3.553	89.7482	104.1	-12.9481
6	82.1000	96.9481	3.553	89.7482	104.1	-14.8481
7	84.1000	96.9481	3.553	89.7482	104.1	-12.8481
8	86.0000	96.9481	3.553	89.7482	104.1	-10.9481
9	86.5000	96.9481	3.553	89.7482	104.1	-10.4481
10	.	96.9481	3.553	89.7482	104.1	.
11	.	96.9481	3.553	89.7482	104.1	.
12	.	96.9481	3.553	89.7482	104.1	.
13	.	96.9481	3.553	89.7482	104.1	.
14	.	96.9481	3.553	89.7482	104.1	.
15	.	96.9481	3.553	89.7482	104.1	.
16	81.5000	96.9481	3.553	89.7482	104.1	-15.4481
17	97.7000	96.9481	3.553	89.7482	104.1	0.7519
18	94.1000	80.0679	2.231	75.5479	84.5879	14.0321
19	.	80.0679	2.231	75.5479	84.5879	.
20	77.8000	80.0679	2.231	75.5479	84.5879	-2.2679
21	79.5000	80.0679	2.231	75.5479	84.5879	-0.5679
22	92.3000	80.0679	2.231	75.5479	84.5879	12.2321
23	.	80.0679	2.231	75.5479	84.5879	.
24	77.9000	80.0679	2.231	75.5479	84.5879	-2.1679
25	.	80.0679	2.231	75.5479	84.5879	.
26	90.9000	80.0679	2.231	75.5479	84.5879	10.8321
27	83.3000	80.0679	2.231	75.5479	84.5879	3.2321
28	74.5000	80.0679	2.231	75.5479	84.5879	-5.5679
29	94.9000	80.0679	2.231	75.5479	84.5879	14.8321
30	.	80.0679	2.231	75.5479	84.5879	.
31	86.1000	80.0679	2.231	75.5479	84.5879	6.0321
32	74.3000	64.9339	2.090	60.6983	69.1696	9.3661
33	89.5000	64.9339	2.090	60.6983	69.1696	24.5661

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 10
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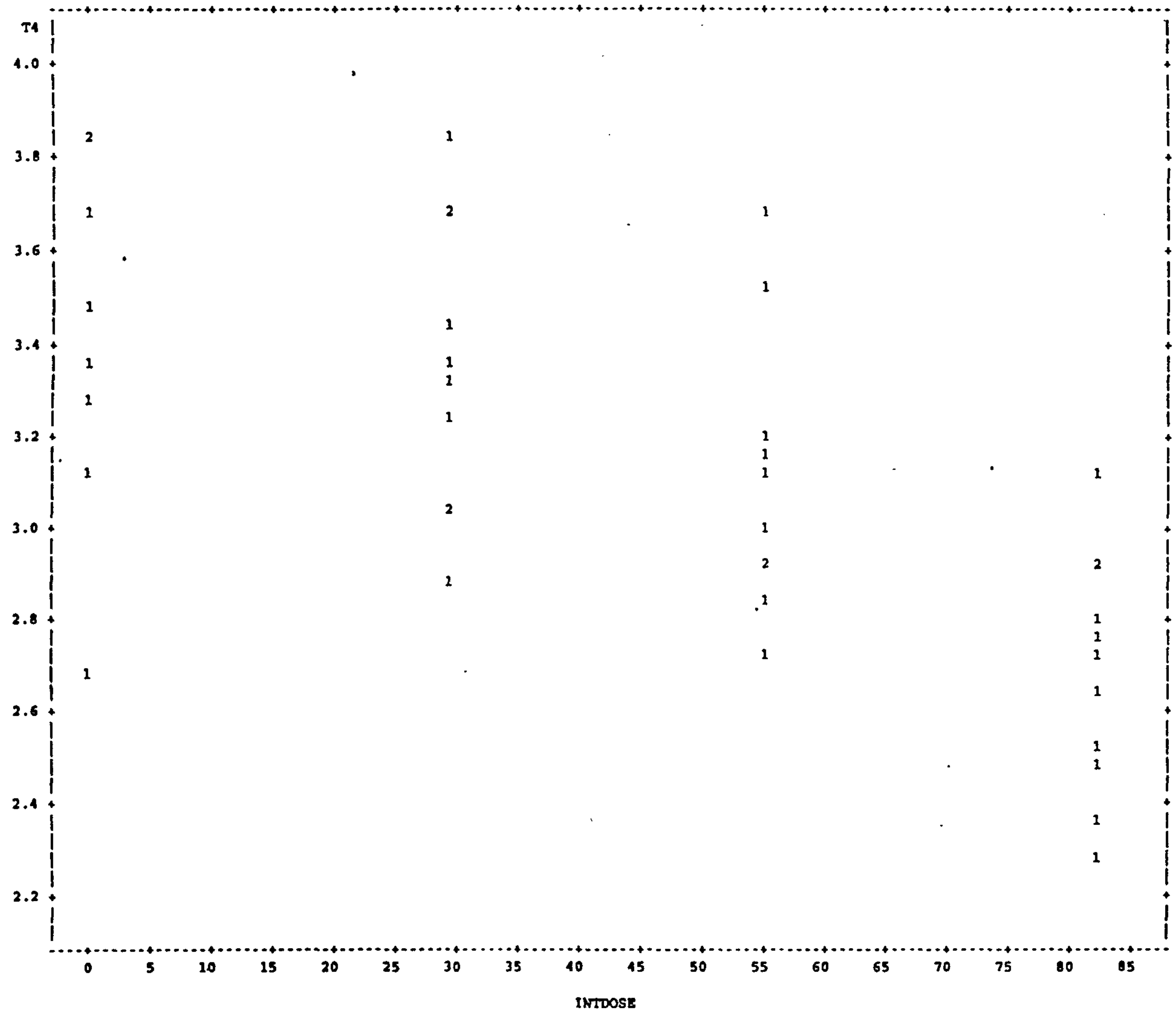
Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

7	4.1900	4.5069	0.135	4.2328	4.7811	-0.3169
8	4.9400	4.5069	0.135	4.2328	4.7811	0.4331
9	4.0900	4.5069	0.135	4.2328	4.7811	-0.4169
10	4.1500	4.5069	0.135	4.2328	4.7811	-0.3569
11	4.3000	4.5069	0.135	4.2328	4.7811	-0.2069
12	4.7200	4.5069	0.135	4.2328	4.7811	0.2131

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
13	4.3400	4.5069	0.135	4.2328	4.7811	-0.1669
14	4.9400	4.5069	0.135	4.2328	4.7811	0.4331
15	5.4600	4.5069	0.135	4.2328	4.7811	0.9531
16	5.5300	4.5069	0.135	4.2328	4.7811	1.0231
17	4.4500	4.5069	0.135	4.2328	4.7811	-0.0569
18	4.6000	4.6240	0.085	4.4518	4.7961	-0.0240
19	3.8700	4.6240	0.085	4.4518	4.7961	-0.7540
20	5.0100	4.6240	0.085	4.4518	4.7961	0.3860
21	3.8800	4.6240	0.085	4.4518	4.7961	-0.7440
22	4.1900	4.6240	0.085	4.4518	4.7961	-0.4340
23	4.6200	4.6240	0.085	4.4518	4.7961	-0.00396
24	4.9400	4.6240	0.085	4.4518	4.7961	0.3160
25	4.7100	4.6240	0.085	4.4518	4.7961	0.0860
26	4.2000	4.6240	0.085	4.4518	4.7961	-0.4240
27	4.0900	4.6240	0.085	4.4518	4.7961	-0.5340
28	4.3000	4.6240	0.085	4.4518	4.7961	-0.3240
29	4.7600	4.6240	0.085	4.4518	4.7961	0.1360
30	4.8900	4.6240	0.085	4.4518	4.7961	0.2660
31	5.2500	4.6240	0.085	4.4518	4.7961	0.6260
32	4.5600	4.7289	0.080	4.5676	4.8902	-0.1689
33	4.7400	4.7289	0.080	4.5676	4.8902	0.0111
34	4.0400	4.7289	0.080	4.5676	4.8902	-0.6889
35	5.1300	4.7289	0.080	4.5676	4.8902	0.4011
36	4.4000	4.7289	0.080	4.5676	4.8902	-0.3289
37	4.5100	4.7289	0.080	4.5676	4.8902	-0.2189
38	4.3900	4.7289	0.080	4.5676	4.8902	-0.3389
39	4.3800	4.7289	0.080	4.5676	4.8902	-0.3489
40	4.8900	4.7289	0.080	4.5676	4.8902	0.1611
41	4.2200	4.7289	0.080	4.5676	4.8902	-0.5089
42	5.6100	4.7289	0.080	4.5676	4.8902	0.8811
43	4.9800	4.7289	0.080	4.5676	4.8902	0.2511
44	5.6300	4.7289	0.080	4.5676	4.8902	0.9011
45	4.7800	4.7289	0.080	4.5676	4.8902	0.0511
46	5.2800	4.7289	0.080	4.5676	4.8902	0.5511
47	4.0300	4.7289	0.080	4.5676	4.8902	-0.6989
48	4.8800	4.7289	0.080	4.5676	4.8902	0.1511
49	5.1100	4.7289	0.080	4.5676	4.8902	0.3811


T4
4.0
3.8
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0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
INTDOSE

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 15
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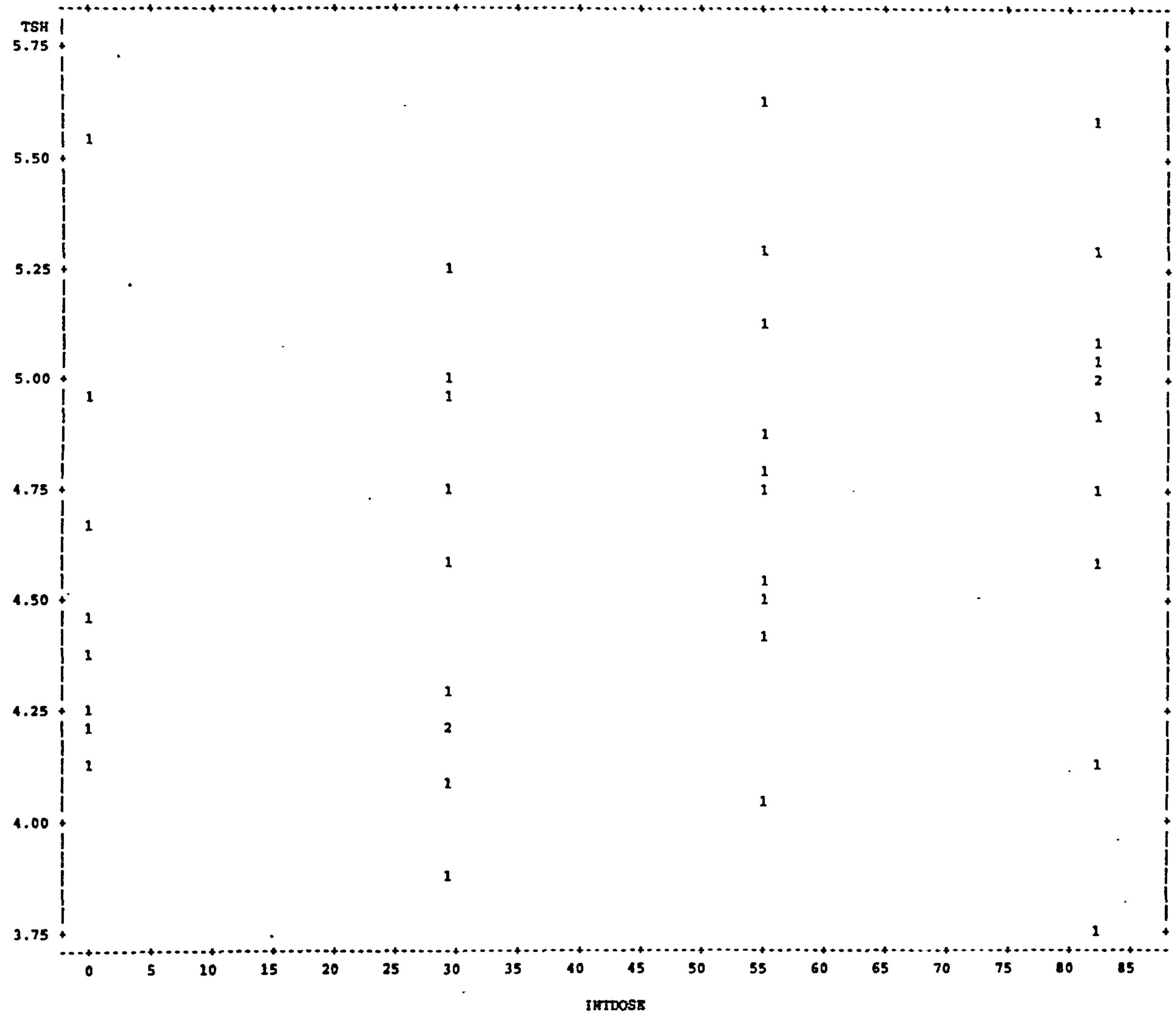

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Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.53614	0.53614	2.420	0.1283
Error	37	8.19845	0.22158		
C Total	38	8.73459			

Root MSE	0.47072	R-square	0.0614
Dep Mean	4.68718	Adj R-sq	0.0360
C.V.	10.04277		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.580047	0.10210248	44.857	0.0001
DCORR	1	0.094958	0.06104586	1.556	0.1283

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Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	3.1700	3.3731	0.067	3.2382	3.5080	-0.2031
2	2.6700	3.3731	0.067	3.2382	3.5080	-0.7031
3	3.4900	3.3731	0.067	3.2382	3.5080	0.1169
4	.	3.3731	0.067	3.2382	3.5080	.
5	3.3700	3.3731	0.067	3.2382	3.5080	-0.00309
6	3.6700	3.3731	0.067	3.2382	3.5080	0.2969
7	3.8300	3.3731	0.067	3.2382	3.5080	0.4569
8	3.2900	3.3731	0.067	3.2382	3.5080	-0.0831
9	.	3.3731	0.067	3.2382	3.5080	.
10	3.0700	3.3731	0.067	3.2382	3.5080	-0.3031
11	2.9000	3.3731	0.067	3.2382	3.5080	-0.4731
12	3.5200	3.3731	0.067	3.2382	3.5080	0.1469
13	3.8100	3.3731	0.067	3.2382	3.5080	0.4369
14	3.8500	3.3731	0.067	3.2382	3.5080	0.4769
15	3.5400	3.3731	0.067	3.2382	3.5080	0.1669
16	3.8500	3.3731	0.067	3.2382	3.5080	0.4769

60	2.7600	2.6680	0.089	2.4872	2.8489	0.0920
61	2.9000	2.6680	0.089	2.4872	2.8489	0.2320
62	.	2.6680	0.089	2.4872	2.8489	.
63	.	2.6680	0.089	2.4872	2.8489	.
64	.	2.6680	0.089	2.4872	2.8489	.
65	2.6400	2.6680	0.089	2.4872	2.8489	-0.0280
66	.	2.6680	0.089	2.4872	2.8489	.

Sum of Residuals	0
Sum of Squared Residuals	3.4866
Predicted Resid SS (Press)	3.8525

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	.	89.2214	1.526	86.1300	92.3128	.
2	88.8000	89.2214	1.526	86.1300	92.3128	-0.4214
3	91.1000	89.2214	1.526	86.1300	92.3128	1.8786
4	97.9000	89.2214	1.526	86.1300	92.3128	8.6786
5	84.0000	89.2214	1.526	86.1300	92.3128	-5.2214
6	82.1000	89.2214	1.526	86.1300	92.3128	-7.1214
7	84.1000	89.2214	1.526	86.1300	92.3128	-5.1214
8	86.0000	89.2214	1.526	86.1300	92.3128	-3.2214
9	86.5000	89.2214	1.526	86.1300	92.3128	-2.7214
10	.	89.2214	1.526	86.1300	92.3128	.
11	.	89.2214	1.526	86.1300	92.3128	.
12	.	89.2214	1.526	86.1300	92.3128	.
13	.	89.2214	1.526	86.1300	92.3128	.
14	.	89.2214	1.526	86.1300	92.3128	.
15	.	89.2214	1.526	86.1300	92.3128	.
16	81.5000	89.2214	1.526	86.1300	92.3128	-7.7214
17	97.7000	89.2214	1.526	86.1300	92.3128	8.4786
18	94.1000	87.6018	1.466	84.6319	90.5717	6.4982
19	.	87.6018	1.466	84.6319	90.5717	.
20	77.8000	87.6018	1.466	84.6319	90.5717	-9.8018
21	79.5000	87.6018	1.466	84.6319	90.5717	-8.1018
22	92.3000	87.6018	1.466	84.6319	90.5717	4.6982
23	.	87.6018	1.466	84.6319	90.5717	.
24	77.9000	87.6018	1.466	84.6319	90.5717	-9.7018
25	.	87.6018	1.466	84.6319	90.5717	.
26	90.9000	87.6018	1.466	84.6319	90.5717	3.2982
27	83.3000	87.6018	1.466	84.6319	90.5717	-4.3018
28	74.5000	87.6018	1.466	84.6319	90.5717	-13.1018
29	94.9000	87.6018	1.466	84.6319	90.5717	7.2982
30	.	87.6018	1.466	84.6319	90.5717	.
31	86.1000	87.6018	1.466	84.6319	90.5717	-1.5018
32	74.3000	73.0252	1.132	70.7307	75.3196	1.2748
33	89.5000	73.0252	1.132	70.7307	75.3196	16.4748

2	4.2300	4.5800	0.102	4.3732	4.7869	-0.3500
3	4.3600	4.5800	0.102	4.3732	4.7869	-0.2200
4	4.0600	4.5800	0.102	4.3732	4.7869	-0.5200
5	4.1200	4.5800	0.102	4.3732	4.7869	-0.4600
6	4.6800	4.5800	0.102	4.3732	4.7869	0.1000
7	4.1900	4.5800	0.102	4.3732	4.7869	-0.3900
8	4.9400	4.5800	0.102	4.3732	4.7869	0.3600
9	4.0900	4.5800	0.102	4.3732	4.7869	-0.4900
10	4.1500	4.5800	0.102	4.3732	4.7869	-0.4300
11	4.3000	4.5800	0.102	4.3732	4.7869	-0.2800
12	4.7200	4.5800	0.102	4.3732	4.7869	0.1400

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
13	4.3400	4.5800	0.102	4.3732	4.7869	-0.2400
14	4.9400	4.5800	0.102	4.3732	4.7869	0.3600
15	5.4600	4.5800	0.102	4.3732	4.7869	0.8800
16	5.5300	4.5800	0.102	4.3732	4.7869	0.9500
17	4.4500	4.5800	0.102	4.3732	4.7869	-0.1300
18	4.6000	4.5895	0.098	4.3908	4.7883	0.0105
19	3.8700	4.5895	0.098	4.3908	4.7883	-0.7195
20	5.0100	4.5895	0.098	4.3908	4.7883	0.4205
21	3.8800	4.5895	0.098	4.3908	4.7883	-0.7095
22	4.1900	4.5895	0.098	4.3908	4.7883	-0.3995
23	4.6200	4.5895	0.098	4.3908	4.7883	0.0305
24	4.9400	4.5895	0.098	4.3908	4.7883	0.3505
25	4.7100	4.5895	0.098	4.3908	4.7883	0.1205
26	4.2000	4.5895	0.098	4.3908	4.7883	-0.3895
27	4.0900	4.5895	0.098	4.3908	4.7883	-0.4995
28	4.3000	4.5895	0.098	4.3908	4.7883	-0.2895
29	4.7600	4.5895	0.098	4.3908	4.7883	0.1705
30	4.8900	4.5895	0.098	4.3908	4.7883	0.3005
31	5.2500	4.5895	0.098	4.3908	4.7883	0.6605
32	4.5600	4.6750	0.076	4.5215	4.8286	-0.1150
33	4.7400	4.6750	0.076	4.5215	4.8286	0.0650
34	4.0400	4.6750	0.076	4.5215	4.8286	-0.6350
35	5.1300	4.6750	0.076	4.5215	4.8286	0.4550
36	4.4000	4.6750	0.076	4.5215	4.8286	-0.2750
37	4.5100	4.6750	0.076	4.5215	4.8286	-0.1650
38	4.3900	4.6750	0.076	4.5215	4.8286	-0.2850
39	4.3800	4.6750	0.076	4.5215	4.8286	-0.2950
40	4.8900	4.6750	0.076	4.5215	4.8286	0.2150
41	4.2200	4.6750	0.076	4.5215	4.8286	-0.4550
42	5.6100	4.6750	0.076	4.5215	4.8286	0.9350
43	4.9800	4.6750	0.076	4.5215	4.8286	0.3050
44	5.6300	4.6750	0.076	4.5215	4.8286	0.9550

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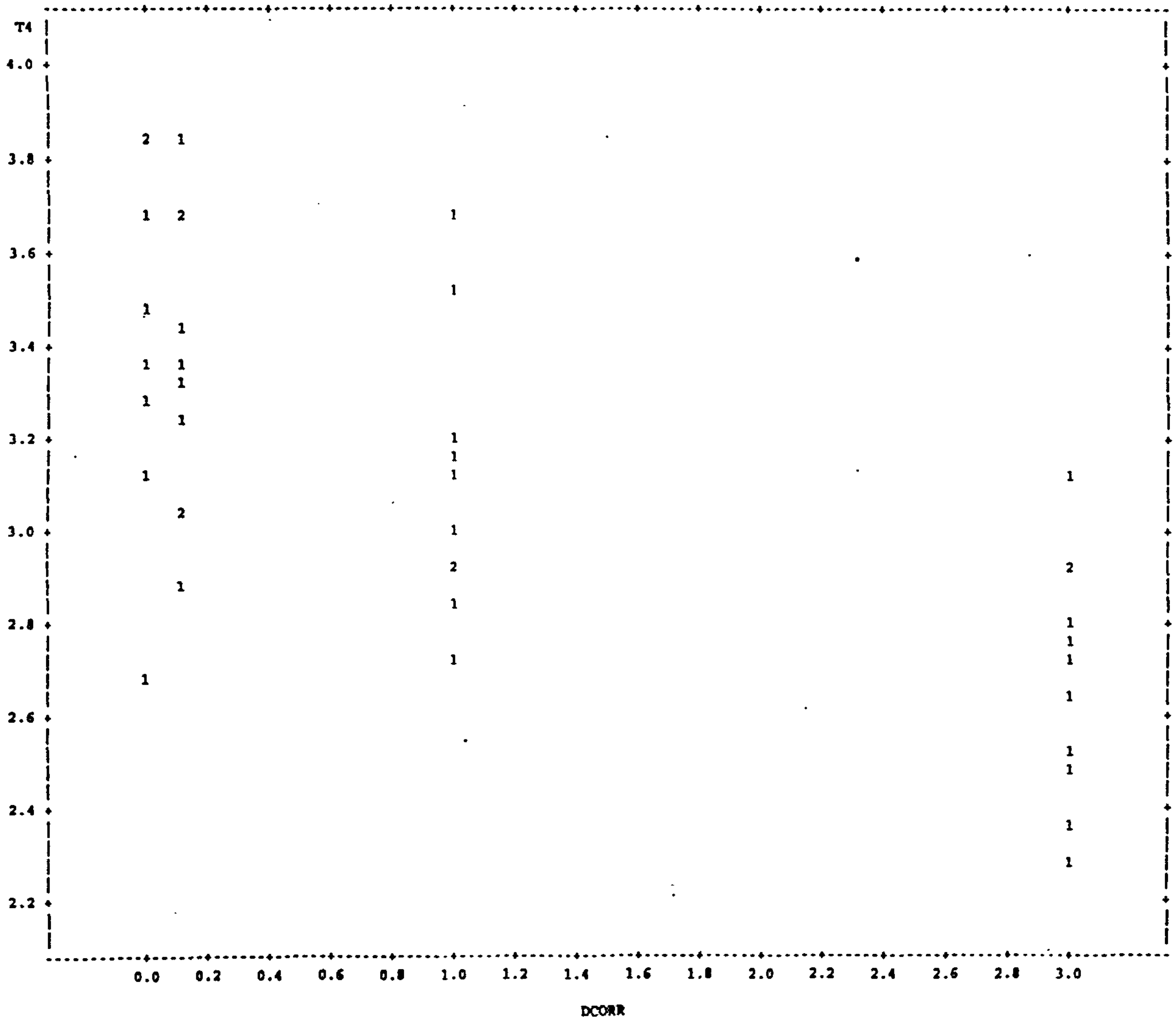
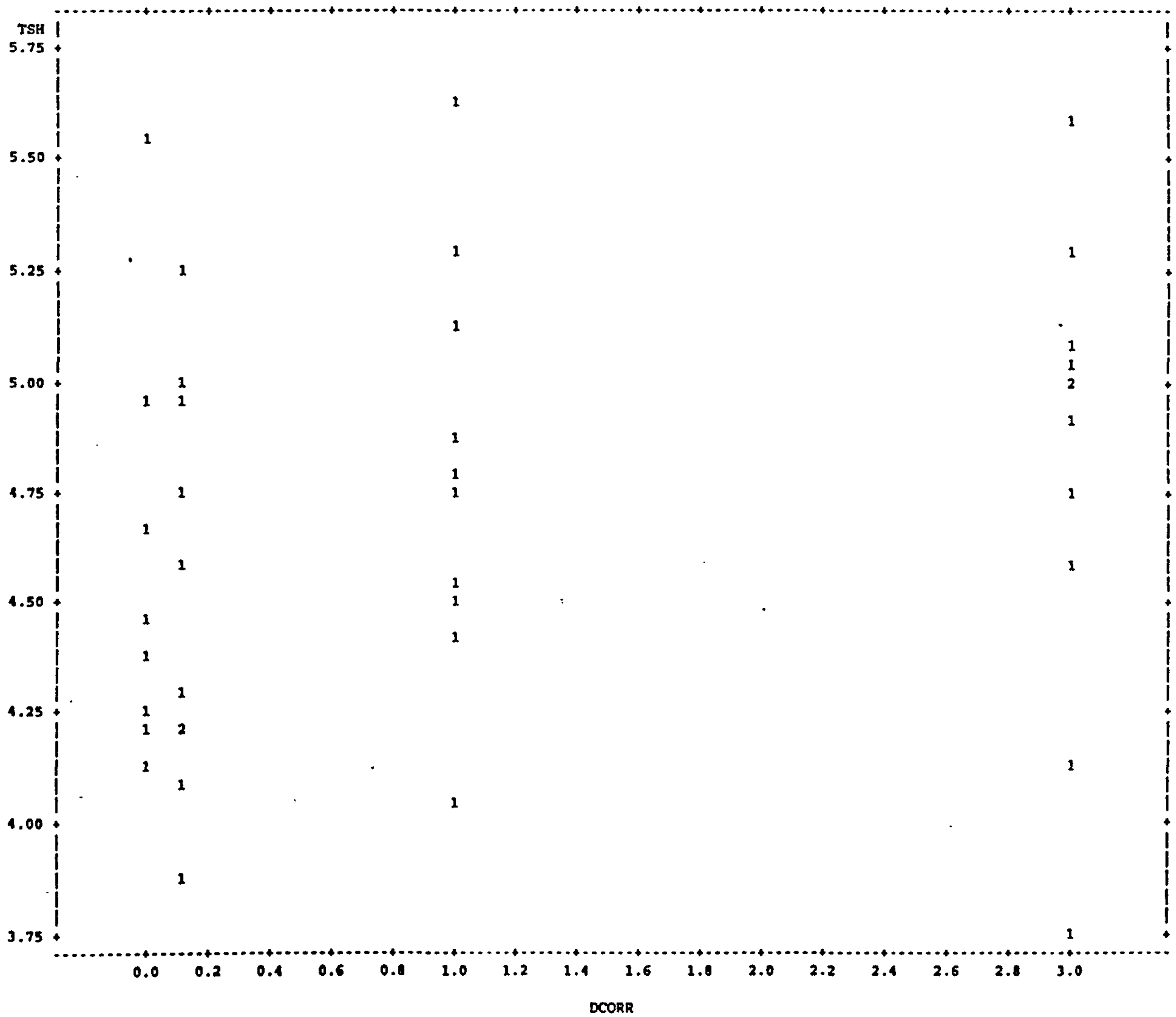



TSH
5.75
5.50
5.25
5.00
4.75
4.50
4.25
4.00
3.75
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0
DCORR

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DCORR	59	0.325424	0.468929	19.200000	0	1.000000
INTDOSE	59	21.440678	23.770651	1265.000000	0	55.000000
T4	59	4.018644	0.774731	237.100000	2.790000	6.350000
T3	59	149.588475	26.736364	8825.720000	103.330000	215.330000
TSH	59	17.151525	1.802690	1011.940000	13.650000	21.520000

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 59

	DCORR	INTDOSE	T4	T3	TSH
DCORR	1.00000	0.98303	-0.61935	-0.71706	0.47101
	0.0	0.0001	0.0001	0.0001	0.0002
INTDOSE	0.98303	1.00000	-0.68767	-0.77399	0.48249
	0.0001	0.0	0.0001	0.0001	0.0001
T4	-0.61935	-0.68767	1.00000	0.69366	-0.31694
	0.0001	0.0001	0.0	0.0001	0.0145
T3	-0.71706	-0.77399	0.69366	1.00000	-0.45130
	0.0001	0.0001	0.0001	0.0	0.0003
TSH	0.47101	0.48249	-0.31694	-0.45130	1.00000
	0.0002	0.0001	0.0145	0.0003	0.0

-- TMPT=15-18 --

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.46317	0.46317	0.951	0.3340
Error	51	24.83270	0.48692		
C Total	52	25.29587			

Root MSE	0.69779	R-square	0.0183
Dep Mean	4.74208	Adj R-sq	-0.0009

Root MSE	2.95180	R-square	0.2018
Dep Mean	13.86491	Adj R-sq	0.1861
C.V.	21.28975		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	12.453554	0.56473417	22.052	0.0001
INTDOSE	1	0.059132	0.01646970	3.590	0.0007

---------- TMPT=15-18 ----------

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	5.1000	4.8327	0.134	4.5647	5.1007	0.2673
2	5.6400	4.8327	0.134	4.5647	5.1007	0.8073
3	6.6400	4.8327	0.134	4.5647	5.1007	1.8073
4	5.8800	4.8327	0.134	4.5647	5.1007	1.0473
5	4.8400	4.8327	0.134	4.5647	5.1007	0.00729
6	5.4200	4.8327	0.134	4.5647	5.1007	0.5873
7	5.2900	4.8327	0.134	4.5647	5.1007	0.4573
8	5.1900	4.8327	0.134	4.5647	5.1007	0.3573
9	5.1000	4.7909	0.108	4.5738	5.0081	0.3091
10	4.2300	4.7909	0.108	4.5738	5.0081	-0.5609
11	5.2100	4.7909	0.108	4.5738	5.0081	0.4191
12	6.0300	4.7909	0.108	4.5738	5.0081	1.2391
13	5.7000	4.7909	0.108	4.5738	5.0081	0.9091
14	5.5800	4.6239	0.155	4.3136	4.9341	0.9561
15	4.8400	4.6239	0.155	4.3136	4.9341	0.2161
16	5.1700	4.6239	0.155	4.3136	4.9341	0.5461
17	4.6900	4.6239	0.155	4.3136	4.9341	0.0661
18	4.9200	4.6239	0.155	4.3136	4.9341	0.2961
19	6.0600	4.6239	0.155	4.3136	4.9341	1.4361
20	4.8100	4.6239	0.155	4.3136	4.9341	0.1861
21	4.8600	4.6239	0.155	4.3136	4.9341	0.2361
22	5.2400	4.6239	0.155	4.3136	4.9341	0.6161
23	5.7400	4.6239	0.155	4.3136	4.9341	1.1161
24	4.8900	4.8327	0.134	4.5647	5.1007	0.0573
25	4.2300	4.8327	0.134	4.5647	5.1007	-0.6027
26	4.4500	4.8327	0.134	4.5647	5.1007	-0.3827
27	4.3300	4.8327	0.134	4.5647	5.1007	-0.5027
28	4.8700	4.8327	0.134	4.5647	5.1007	0.0373
29	3.4700	4.8327	0.134	4.5647	5.1007	-1.3627

9	173.2	150.2	3.865	142.5	158.0	23.0172
10	170.5	150.2	3.865	142.5	158.0	20.2772
11	154.8	150.2	3.865	142.5	158.0	4.5972
12	136.3	150.2	3.865	142.5	158.0	-13.9128
13	183.3	150.2	3.865	142.5	158.0	33.0872
14	122.4	123.5	5.522	112.4	134.6	-1.0998
15	130.7	123.5	5.522	112.4	134.6	7.2202
16	139.6	123.5	5.522	112.4	134.6	16.0702
17	137.4	123.5	5.522	112.4	134.6	13.9502
18	133.2	123.5	5.522	112.4	134.6	9.6902
19	101.6	123.5	5.522	112.4	134.6	-21.8798
20	125.9	123.5	5.522	112.4	134.6	2.4202
21	109.9	123.5	5.522	112.4	134.6	-13.5598
22	124.6	123.5	5.522	112.4	134.6	1.0802
23	112.3	123.5	5.522	112.4	134.6	-11.2198
24	133.3	156.9	4.770	147.3	166.5	-23.6135
25	147.0	156.9	4.770	147.3	166.5	-9.9135
26	148.5	156.9	4.770	147.3	166.5	-8.3735
27	113.0	156.9	4.770	147.3	166.5	-43.8835
28	141.8	156.9	4.770	147.3	166.5	-15.1035
29	115.1	156.9	4.770	147.3	166.5	-41.7735
30	125.5	156.9	4.770	147.3	166.5	-31.4335
31	155.3	156.9	4.770	147.3	166.5	-1.6135
32	117.3	156.9	4.770	147.3	166.5	-39.6235
33	133.2	156.9	4.770	147.3	166.5	-23.6535
34	144.3	150.2	3.865	142.5	158.0	-5.8928
35	134.1	150.2	3.865	142.5	158.0	-16.0728
36	134.2	150.2	3.865	142.5	158.0	-16.0528
37	134.9	150.2	3.865	142.5	158.0	-15.3228
38	142.6	150.2	3.865	142.5	158.0	-7.6628
39	113.0	150.2	3.865	142.5	158.0	-37.2128
40	147.2	150.2	3.865	142.5	158.0	-2.9728
41	128.8	150.2	3.865	142.5	158.0	-21.3728
42	145.9	150.2	3.865	142.5	158.0	-4.3028

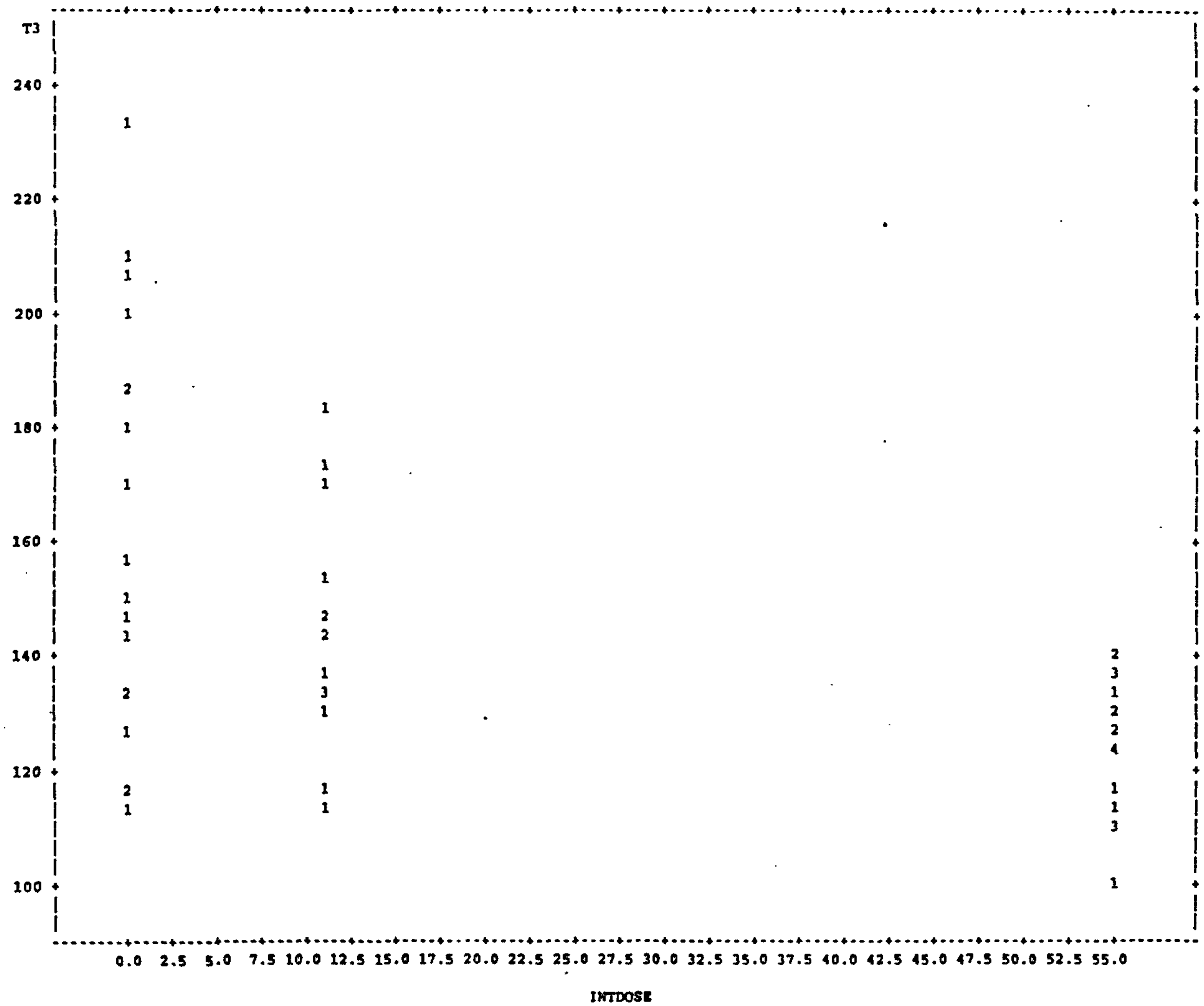
---------- TMPT=15-18 ----------

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
43	115.3	150.2	3.865	142.5	158.0	-34.9228
44	140.0	123.5	5.522	112.4	134.6	16.4602
45	122.8	123.5	5.522	112.4	134.6	-0.7098
46	109.9	123.5	5.522	112.4	134.6	-13.5598
47	121.8	123.5	5.522	112.4	134.6	-1.6898
48	117.3	123.5	5.522	112.4	134.6	-6.1998
49	127.9	123.5	5.522	112.4	134.6	4.3902

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
33	11.3600	12.4536	0.565	11.3198	13.5873	-1.0936
34	11.8600	13.1040	0.458	12.1855	14.0225	-1.2440
35	11.8300	13.1040	0.458	12.1855	14.0225	-1.2740
36	10.3300	13.1040	0.458	12.1855	14.0225	-2.7740
37	10.0300	13.1040	0.458	12.1855	14.0225	-3.0740
38	13.4700	13.1040	0.458	12.1855	14.0225	0.3660
39	10.7800	13.1040	0.458	12.1855	14.0225	-2.3240
40	13.7100	13.1040	0.458	12.1855	14.0225	0.6060
41	11.4600	13.1040	0.458	12.1855	14.0225	-1.6440
42	11.3600	13.1040	0.458	12.1855	14.0225	-1.7440
43	12.3500	13.1040	0.458	12.1855	14.0225	-0.7540
44	13.0700	15.7058	0.654	14.3935	17.0181	-2.6358
45	12.3300	15.7058	0.654	14.3935	17.0181	-3.3758
46	10.1300	15.7058	0.654	14.3935	17.0181	-5.5758
47	12.0200	15.7058	0.654	14.3935	17.0181	-3.6858
48	13.4800	15.7058	0.654	14.3935	17.0181	-2.2258
49	11.7000	15.7058	0.654	14.3935	17.0181	-4.0058
50	12.4800	15.7058	0.654	14.3935	17.0181	-3.2258
51	13.1400	15.7058	0.654	14.3935	17.0181	-2.5658
52	14.9100	15.7058	0.654	14.3935	17.0181	-0.7958
53	12.6300	15.7058	0.654	14.3935	17.0181	-3.0758

Sum of Residuals	0
Sum of Squared Residuals	444.3705
Predicted Resid SS (Press)	482.8323

--- TMPT=15-18 ---


T3
240
220
200
180
160
140
120
100
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0 32.5 35.0 37.5 40.0 42.5 45.0 47.5 50.0 52.5 55.0
INTDOSE

-- TMPT=92-95 --

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	16.46231	16.46231	51.137	0.0001
Error	57	18.34978	0.32193		
C Total	58	34.81209			

Root MSE	0.56739	R-square	0.4729
Dep Mean	4.01864	Adj R-sq	0.4636
C.V.	14.11882		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.499183	0.09986014	45.055	0.0001
INTDOSE	1	-0.022413	0.00313417	-7.151	0.0001

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	24837.40430	24837.40430	85.167	0.0001
Error	57	16622.91907	291.63016		
C Total	58	41460.32336			

Root MSE	17.07718	R-square	0.5991
Dep Mean	149.58847	Adj R-sq	0.5920
C.V.	11.41611		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|

11	3.2200	4.2526	0.081	4.0909	4.4144	-1.0326
12	4.2200	4.2526	0.081	4.0909	4.4144	-0.0326
13	4.7000	4.2526	0.081	4.0909	4.4144	0.4474
14	4.2300	4.2526	0.081	4.0909	4.4144	-0.0226
15	4.1200	4.2526	0.081	4.0909	4.4144	-0.1326
16	4.7600	4.2526	0.081	4.0909	4.4144	0.5074
17	4.3800	4.2526	0.081	4.0909	4.4144	0.1274
18	4.4900	4.2526	0.081	4.0909	4.4144	0.2374
19	4.6500	4.2526	0.081	4.0909	4.4144	0.3974
20	4.7900	4.2526	0.081	4.0909	4.4144	0.5374
21	3.8700	3.2665	0.129	3.0091	3.5239	0.6035
22	3.1400	3.2665	0.129	3.0091	3.5239	-0.1265
23	3.8400	3.2665	0.129	3.0091	3.5239	0.5735
24	3.3200	3.2665	0.129	3.0091	3.5239	0.0535
25	3.5900	3.2665	0.129	3.0091	3.5239	0.3235
26	2.7900	3.2665	0.129	3.0091	3.5239	-0.4765
27	3.2000	3.2665	0.129	3.0091	3.5239	-0.0665
28	3.6100	3.2665	0.129	3.0091	3.5239	0.3435
29	3.8200	3.2665	0.129	3.0091	3.5239	0.5535
30	3.4900	3.2665	0.129	3.0091	3.5239	0.2235
31	3.7300	4.4992	0.100	4.2992	4.6991	-0.7692
32	4.3900	4.4992	0.100	4.2992	4.6991	-0.1092
33	4.1100	4.4992	0.100	4.2992	4.6991	-0.3892
34	4.3800	4.4992	0.100	4.2992	4.6991	-0.1192
35	4.7000	4.4992	0.100	4.2992	4.6991	0.2008
36	4.1000	4.4992	0.100	4.2992	4.6991	-0.3992
37	5.2800	4.4992	0.100	4.2992	4.6991	0.7808
38	4.7500	4.4992	0.100	4.2992	4.6991	0.2508
39	4.2500	4.4992	0.100	4.2992	4.6991	-0.2492
40	4.7200	4.4992	0.100	4.2992	4.6991	0.2208
41	3.6100	4.2526	0.081	4.0909	4.4144	-0.6426
42	3.7300	4.2526	0.081	4.0909	4.4144	-0.5226
43	3.6000	4.2526	0.081	4.0909	4.4144	-0.6526
44	3.3600	4.2526	0.081	4.0909	4.4144	-0.8926
45	3.1800	4.2526	0.081	4.0909	4.4144	-1.0726
46	3.3000	4.2526	0.081	4.0909	4.4144	-0.9526
47	3.1000	4.2526	0.081	4.0909	4.4144	-1.1526
48	3.4600	4.2526	0.081	4.0909	4.4144	-0.7926
49	4.2900	4.2526	0.081	4.0909	4.4144	0.0374
50	3.5800	4.2526	0.081	4.0909	4.4144	-0.6726
51	3.0200	3.2665	0.129	3.0091	3.5239	-0.2465
52	3.1000	3.2665	0.129	3.0091	3.5239	-0.1665

-- TMPT=92-95 --

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

33	156.3	168.3	3.006	162.2	174.3	-11.9538
34	184.1	168.3	3.006	162.2	174.3	15.7962
35	162.7	168.3	3.006	162.2	174.3	-5.5538
36	174.6	168.3	3.006	162.2	174.3	6.3062

--- TMPT=92-95 ---

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
37	178.4	168.3	3.006	162.2	174.3	10.1262
38	157.3	168.3	3.006	162.2	174.3	-10.9138
39	154.0	168.3	3.006	162.2	174.3	-14.2238
40	148.7	168.3	3.006	162.2	174.3	-19.5338
41	161.1	158.7	2.432	153.8	163.5	2.4223
42	133.7	158.7	2.432	153.8	163.5	-24.9777
43	153.4	158.7	2.432	153.8	163.5	-5.2577
44	140.3	158.7	2.432	153.8	163.5	-18.3877
45	122.9	158.7	2.432	153.8	163.5	-35.7777
46	126.2	158.7	2.432	153.8	163.5	-32.4477
47	136.1	158.7	2.432	153.8	163.5	-22.5777
48	158.9	158.7	2.432	153.8	163.5	0.1723
49	134.9	158.7	2.432	153.8	163.5	-23.7377
50	163.8	158.7	2.432	153.8	163.5	5.1023
51	122.9	120.4	3.868	112.6	128.1	2.4869
52	116.1	120.4	3.868	112.6	128.1	-4.2831
53	116.6	120.4	3.868	112.6	128.1	-3.7831
54	107.7	120.4	3.868	112.6	128.1	-12.6431
55	135.3	120.4	3.868	112.6	128.1	14.9269
56	117.3	120.4	3.868	112.6	128.1	-3.0831
57	137.2	120.4	3.868	112.6	128.1	16.8169
58	124.0	120.4	3.868	112.6	128.1	3.6569
59	125.6	120.4	3.868	112.6	128.1	5.2369

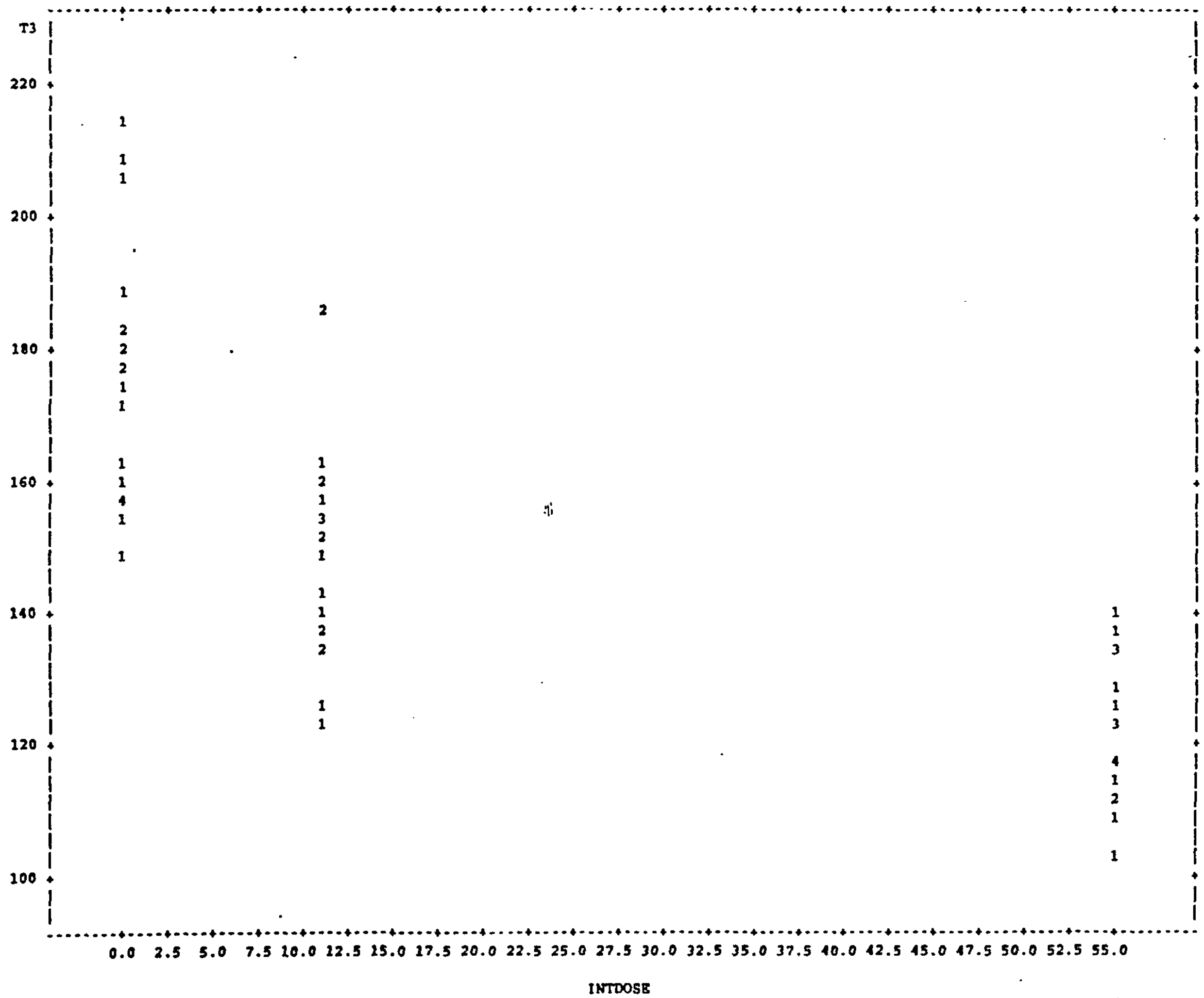
Sum of Residuals	0
Sum of Squared Residuals	16622.9191
Predicted Resid SS (Press)	17636.1185

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	15.7100	16.3670	0.280	15.8057	16.9284	-0.6570
2	14.3800	16.3670	0.280	15.8057	16.9284	-1.9870
3	15.1800	16.3670	0.280	15.8057	16.9284	-1.1870
4	17.7200	16.3670	0.280	15.8057	16.9284	1.3530
5	17.5000	16.3670	0.280	15.8057	16.9284	1.1330

47	15.8900	16.7695	0.227	16.3153	17.2237	-0.8795
48	16.6400	16.7695	0.227	16.3153	17.2237	-0.1295
49	14.4500	16.7695	0.227	16.3153	17.2237	-2.3195
50	15.7300	16.7695	0.227	16.3153	17.2237	-1.0395
51	17.7500	18.3795	0.361	17.6570	19.1020	-0.6295
52	18.8100	18.3795	0.361	17.6570	19.1020	0.4305
53	16.2600	18.3795	0.361	17.6570	19.1020	-2.1195
54	19.2000	18.3795	0.361	17.6570	19.1020	0.8205
55	18.7600	18.3795	0.361	17.6570	19.1020	0.3805
56	15.6900	18.3795	0.361	17.6570	19.1020	-2.6895
57	18.2700	18.3795	0.361	17.6570	19.1020	-0.1095
58	14.9700	18.3795	0.361	17.6570	19.1020	-3.4095
59	18.9300	18.3795	0.361	17.6570	19.1020	0.5505

Sum of Residuals	0
Sum of Squared Residuals	144.6044
Predicted Resid SS (Press)	154.8007

TMPT=92-95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INTDOSE

------------------------------ TMPT=15-18 ------------------------------

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	0.29494	0.29494	0.602	0.4415
Error	51	25.00093	0.49021		
C Total	52	25.29587			

Root MSE	0.70015	R-square	0.0117
Dep Mean	4.74208	Adj R-sq	-0.0077
C.V.	14.76470		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.800851	0.12243807	39.210	0.0001
DCORR	1	-0.154596	0.19930727	-0.776	0.4415

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	10442.75272	10442.75272	16.083	0.0002
Error	51	33114.80266	649.30986		
C Total	52	43557.55538			

Root MSE	25.48156	R-square	0.2397
Dep Mean	142.39755	Adj R-sq	0.2248
C.V.	17.89466		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|

11	5.2100	4.7993	0.121	4.5560	5.0427	0.4107
12	6.0300	4.7993	0.121	4.5560	5.0427	1.2307
13	5.7000	4.7993	0.121	4.5560	5.0427	0.9007
14	5.5800	4.6463	0.157	4.3320	4.9606	0.9337
15	4.8400	4.6463	0.157	4.3320	4.9606	0.1937
16	5.1700	4.6463	0.157	4.3320	4.9606	0.5237
17	4.6900	4.6463	0.157	4.3320	4.9606	0.0437
18	4.9200	4.6463	0.157	4.3320	4.9606	0.2737
19	6.0600	4.6463	0.157	4.3320	4.9606	1.4137
20	4.8100	4.6463	0.157	4.3320	4.9606	0.1637
21	4.8600	4.6463	0.157	4.3320	4.9606	0.2137
22	5.2400	4.6463	0.157	4.3320	4.9606	0.5937
23	5.7400	4.6463	0.157	4.3320	4.9606	1.0937
24	4.8900	4.8009	0.122	4.5550	5.0467	0.0891
25	4.2300	4.8009	0.122	4.5550	5.0467	-0.5709
26	4.4500	4.8009	0.122	4.5550	5.0467	-0.3509
27	4.3300	4.8009	0.122	4.5550	5.0467	-0.4709
28	4.8700	4.8009	0.122	4.5550	5.0467	0.0691
29	3.4700	4.8009	0.122	4.5550	5.0467	-1.3309
30	4.8100	4.8009	0.122	4.5550	5.0467	0.00915
31	4.3600	4.8009	0.122	4.5550	5.0467	-0.4409
32	4.7500	4.8009	0.122	4.5550	5.0467	-0.0509
33	4.7200	4.8009	0.122	4.5550	5.0467	-0.0809
34	4.4800	4.7993	0.121	4.5560	5.0427	-0.3193
35	4.3900	4.7993	0.121	4.5560	5.0427	-0.4093
36	4.5900	4.7993	0.121	4.5560	5.0427	-0.2093
37	4.5800	4.7993	0.121	4.5560	5.0427	-0.2193
38	4.7400	4.7993	0.121	4.5560	5.0427	-0.0593
39	4.6000	4.7993	0.121	4.5560	5.0427	-0.1993
40	3.2400	4.7993	0.121	4.5560	5.0427	-1.5593
41	4.4400	4.7993	0.121	4.5560	5.0427	-0.3593
42	3.8300	4.7993	0.121	4.5560	5.0427	-0.9693
43	4.3400	4.7993	0.121	4.5560	5.0427	-0.4593
44	4.0200	4.6463	0.157	4.3320	4.9606	-0.6263
45	3.4500	4.6463	0.157	4.3320	4.9606	-1.1963
46	4.3000	4.6463	0.157	4.3320	4.9606	-0.3463
47	4.4600	4.6463	0.157	4.3320	4.9606	-0.1863
48	3.6700	4.6463	0.157	4.3320	4.9606	-0.9763
49	3.8100	4.6463	0.157	4.3320	4.9606	-0.8363
50	4.6000	4.6463	0.157	4.3320	4.9606	-0.0463
51	4.6100	4.6463	0.157	4.3320	4.9606	-0.0363
52	4.0500	4.6463	0.157	4.3320	4.9606	-0.5963

-- TMPT=15-18 --

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

39	113.0	153.2	4.412	144.3	162.0	-40.1662
40	147.2	153.2	4.412	144.3	162.0	-5.9262
41	128.8	153.2	4.412	144.3	162.0	-24.3262
42	145.9	153.2	4.412	144.3	162.0	-7.2562

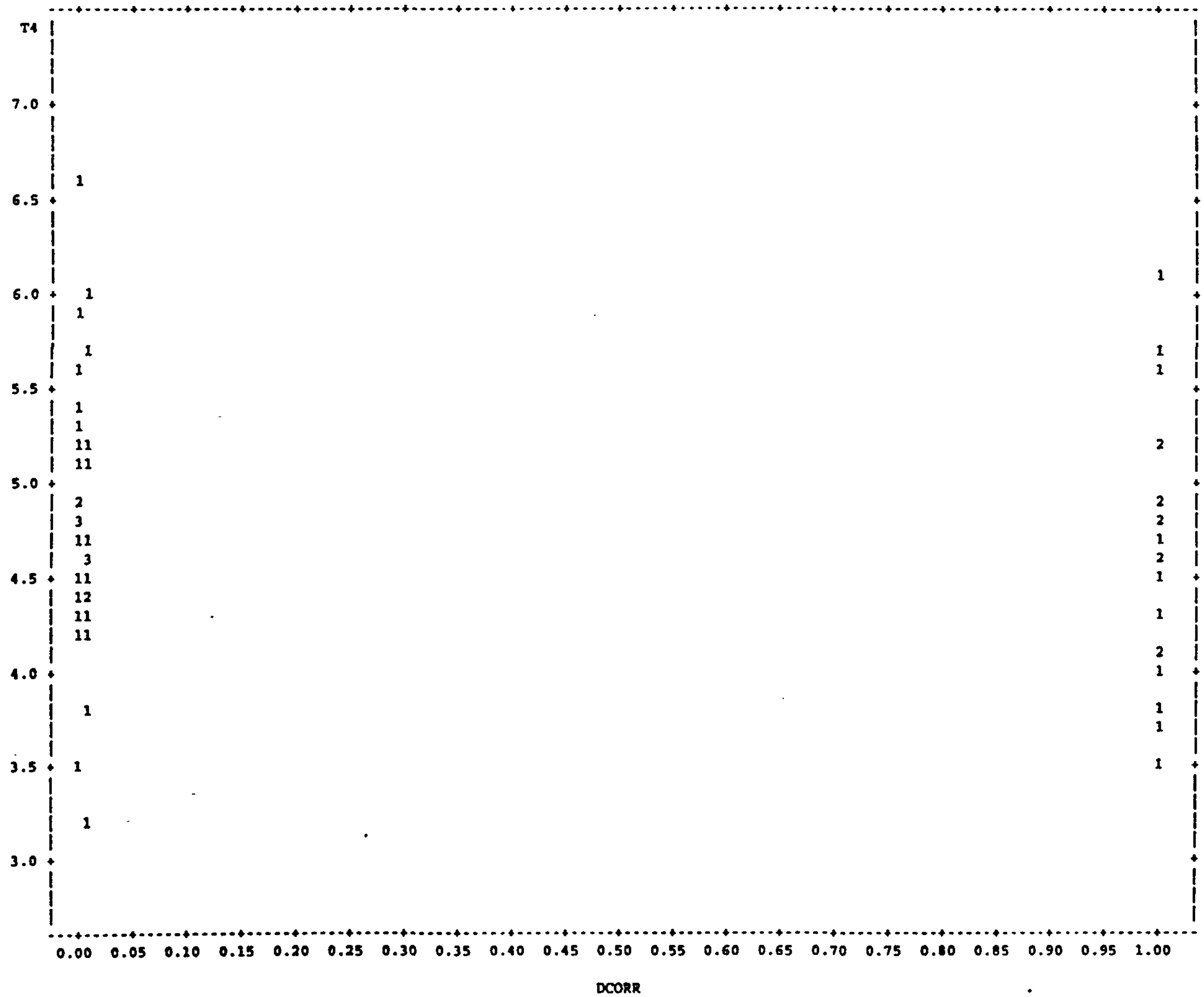
--- TMPT=15-18 ---

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
43	115.3	153.2	4.412	144.3	162.0	-37.8762
44	140.0	124.4	5.698	112.9	135.8	15.5825
45	122.8	124.4	5.698	112.9	135.8	-1.5875
46	109.9	124.4	5.698	112.9	135.8	-14.4375
47	121.8	124.4	5.698	112.9	135.8	-2.5675
48	117.3	124.4	5.698	112.9	135.8	-7.0775
49	127.9	124.4	5.698	112.9	135.8	3.5125
50	138.2	124.4	5.698	112.9	135.8	13.8525
51	108.5	124.4	5.698	112.9	135.8	-15.8675
52	135.8	124.4	5.698	112.9	135.8	11.3925
53	129.1	124.4	5.698	112.9	135.8	4.6925

Sum of Residuals	0
Sum of Squared Residuals	33114.8027
Predicted Resid SS (Press)	35338.4234

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	13.0800	12.7408	0.519	11.6993	13.7822	0.3392
2	13.7200	12.7408	0.519	11.6993	13.7822	0.9792
3	14.2000	12.7408	0.519	11.6993	13.7822	1.4592
4	16.3800	12.7408	0.519	11.6993	13.7822	3.6392
5	13.2100	12.7408	0.519	11.6993	13.7822	0.4692
6	15.5200	12.7408	0.519	11.6993	13.7822	2.7792
7	17.4500	12.7408	0.519	11.6993	13.7822	4.7092
8	15.3000	12.7408	0.519	11.6993	13.7822	2.5592
9	15.2200	12.7703	0.514	11.7393	13.8014	2.4497
10	12.7600	12.7703	0.514	11.7393	13.8014	-0.0103
11	17.6300	12.7703	0.514	11.7393	13.8014	4.8597
12	18.4100	12.7703	0.514	11.7393	13.8014	5.6397
13	16.4900	12.7703	0.514	11.7393	13.8014	3.7197
14	19.9300	15.6976	0.663	14.3659	17.0292	4.2324
15	20.2700	15.6976	0.663	14.3659	17.0292	4.5724
16	17.3900	15.6976	0.663	14.3659	17.0292	1.6924
17	17.0300	15.6976	0.663	14.3659	17.0292	1.3324

TMPT=15-18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3.0
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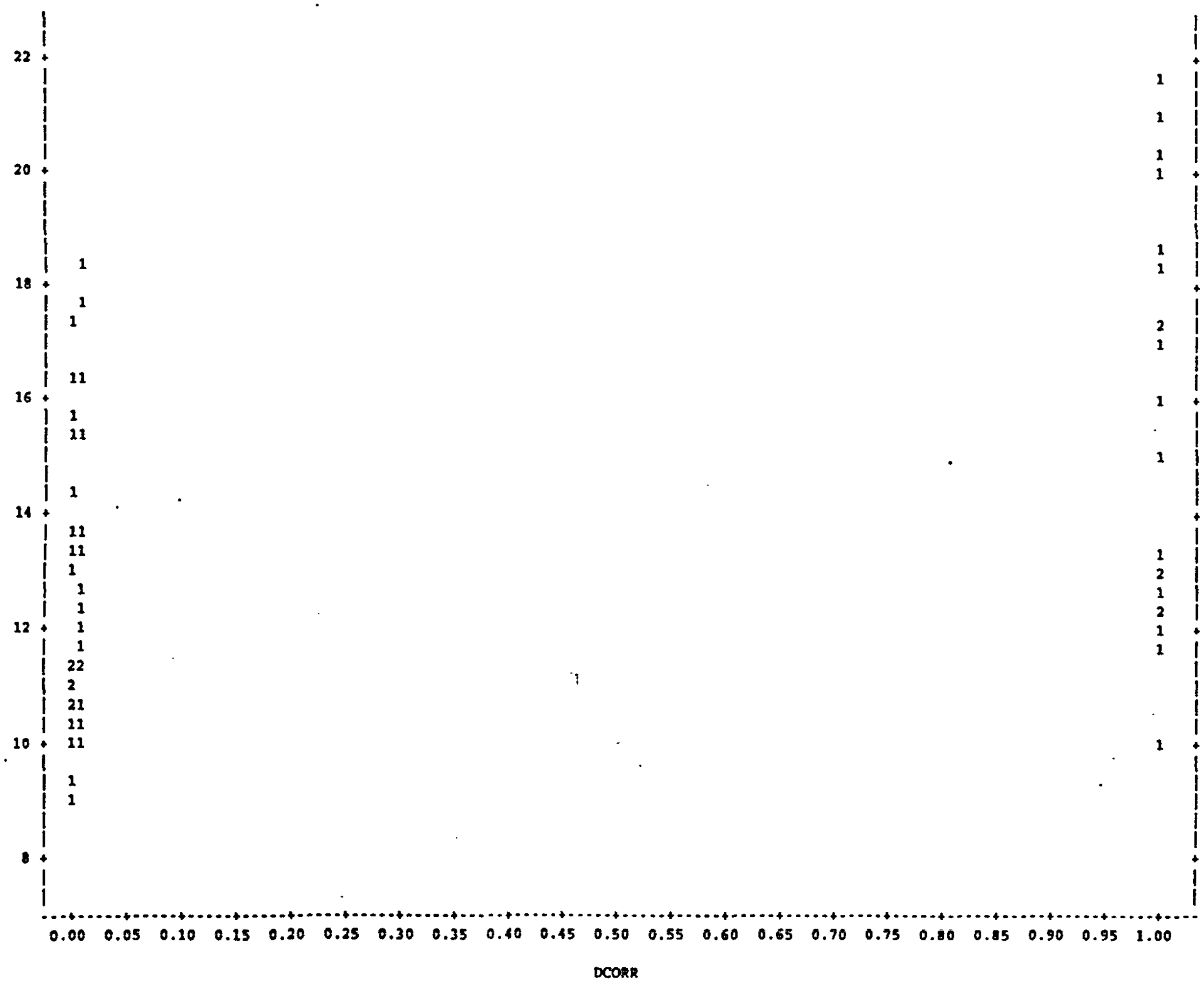
DCORR


22
20
18
16
14
12
10
8
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00
DCORR

INTERCEP	1	162.893104	2.98723098	54.530	0.0001
DCORR	1	-40.884017	5.26374877	-7.767	0.0001

---------- TMPT=92-95 ----------

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	41.81414	41.81414	16.250	0.0002
Error	57	146.66803	2.57312		
C Total	58	188.48216			

Root MSE	1.60410	R-square	0.2218
Dep Mean	17.15153	Adj R-sq	0.2082
C.V.	9.35250		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	16.562289	0.25490776	64.974	0.0001
DCORR	1	1.810676	0.44916861	4.031	0.0002

---------- TMPT=92-95 ----------

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.6100	4.3516	0.098	4.1564	4.5469	0.2584
2	5.0200	4.3516	0.098	4.1564	4.5469	0.6684
3	4.9600	4.3516	0.098	4.1564	4.5469	0.6084
4	4.6300	4.3516	0.098	4.1564	4.5469	0.2784
5	6.3500	4.3516	0.098	4.1564	4.5469	1.9984
6	5.1900	4.3516	0.098	4.1564	4.5469	0.8384
7	5.1200	4.3516	0.098	4.1564	4.5469	0.7684
8	4.3700	4.3516	0.098	4.1564	4.5469	0.0184
9	4.9700	4.3516	0.098	4.1564	4.5469	0.6184
10	5.3800	4.3516	0.098	4.1564	4.5469	1.0284

53	3.4100	3.3284	0.141	3.0465	3.6103	0.0816
54	2.9000	3.3284	0.141	3.0465	3.6103	-0.4284
55	3.7800	3.3284	0.141	3.0465	3.6103	0.4516
56	3.4300	3.3284	0.141	3.0465	3.6103	0.1016
57	3.1800	3.3284	0.141	3.0465	3.6103	-0.1484
58	2.7900	3.3284	0.141	3.0465	3.6103	-0.5384
59	3.0400	3.3284	0.141	3.0465	3.6103	-0.2884

Sum of Residuals	0
Sum of Squared Residuals	21.4585
Predicted Resid SS (Press)	22.7137

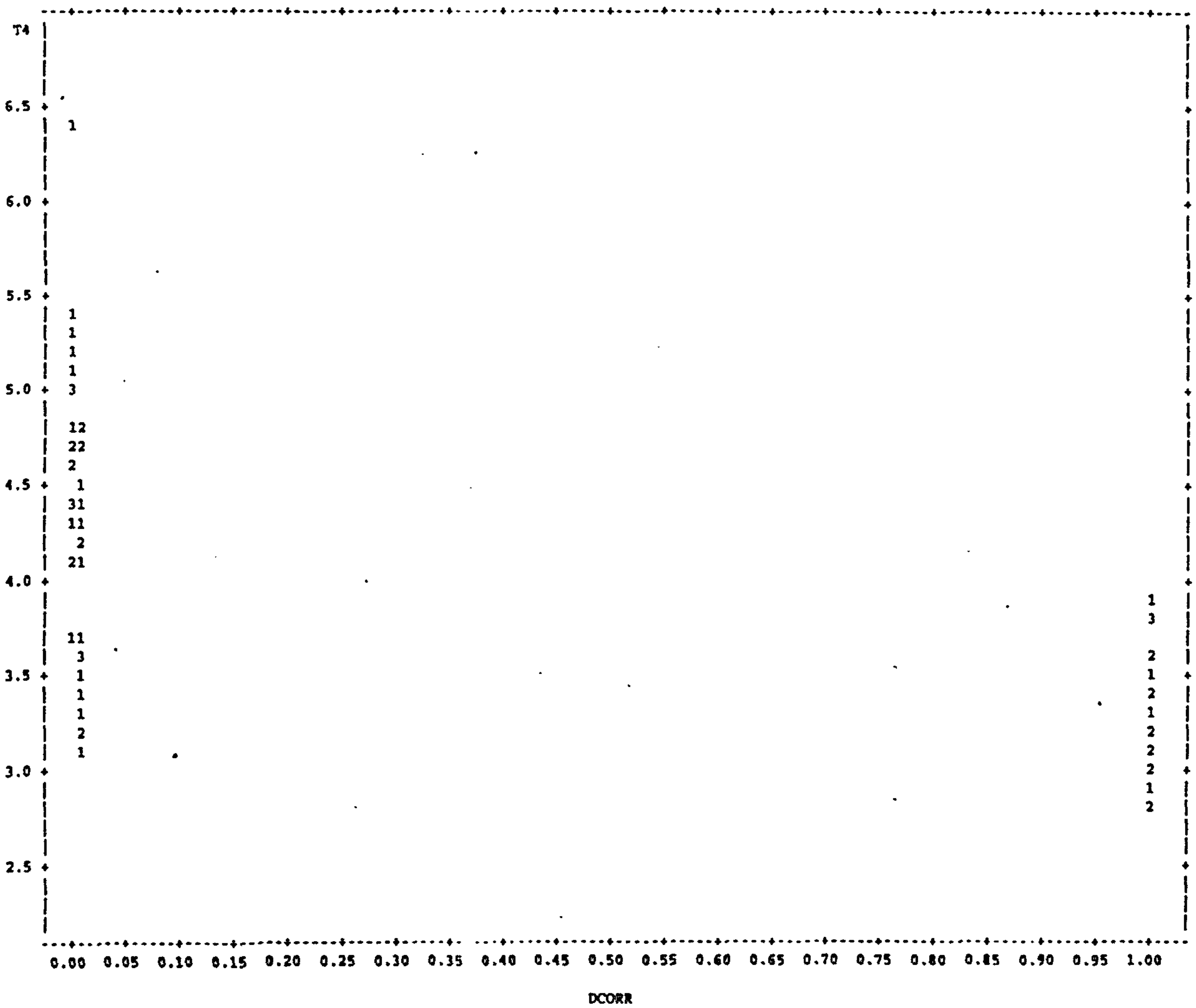
Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	204.7	162.9	2.987	156.9	168.9	41.7969
2	182.7	162.9	2.987	156.9	168.9	19.7569
3	158.2	162.9	2.987	156.9	168.9	-4.6631
4	156.9	162.9	2.987	156.9	168.9	-6.0031
5	189.8	162.9	2.987	156.9	168.9	26.9469
6	179.3	162.9	2.987	156.9	168.9	16.3869
7	215.3	162.9	2.987	156.9	168.9	52.4369
8	160.9	162.9	2.987	156.9	168.9	-2.0331
9	171.1	162.9	2.987	156.9	168.9	8.1969
10	179.5	162.9	2.987	156.9	168.9	16.6169
11	158.2	162.5	2.957	156.6	168.4	-4.3343
12	155.4	162.5	2.957	156.6	168.4	-7.0843
13	136.2	162.5	2.957	156.6	168.4	-26.2943
14	152.6	162.5	2.957	156.6	168.4	-9.8743
15	154.1	162.5	2.957	156.6	168.4	-8.4243
16	144.1	162.5	2.957	156.6	168.4	-18.4243
17	186.9	162.5	2.957	156.6	168.4	24.3757
18	149.5	162.5	2.957	156.6	168.4	-12.9743
19	151.6	162.5	2.957	156.6	168.4	-10.8743
20	186.2	162.5	2.957	156.6	168.4	23.7057
21	134.5	122.0	4.312	113.4	130.6	12.5209
22	133.3	122.0	4.312	113.4	130.6	11.2709
23	116.0	122.0	4.312	113.4	130.6	-6.0291
24	112.2	122.0	4.312	113.4	130.6	-9.8491
25	103.3	122.0	4.312	113.4	130.6	-18.6791
26	112.8	122.0	4.312	113.4	130.6	-9.2291
27	138.7	122.0	4.312	113.4	130.6	16.6809
28	129.2	122.0	4.312	113.4	130.6	7.1809
29	114.3	122.0	4.312	113.4	130.6	-7.7391
30	123.7	122.0	4.312	113.4	130.6	1.7009
31	208.4	162.9	2.987	156.9	168.9	45.5169
32	176.3	162.9	2.987	156.9	168.9	13.4069

6	15.3900	16.5623	0.255	16.0518	17.0727	-1.1723
7	17.6100	16.5623	0.255	16.0518	17.0727	1.0477
8	14.9800	16.5623	0.255	16.0518	17.0727	-1.5823
9	14.7900	16.5623	0.255	16.0518	17.0727	-1.7723
10	18.6100	16.5623	0.255	16.0518	17.0727	2.0477
11	16.2700	16.5804	0.252	16.0751	17.0857	-0.3104
12	16.8700	16.5804	0.252	16.0751	17.0857	0.2896
13	15.9500	16.5804	0.252	16.0751	17.0857	-0.6304
14	17.5400	16.5804	0.252	16.0751	17.0857	0.9596
15	15.4000	16.5804	0.252	16.0751	17.0857	-1.1804
16	15.4600	16.5804	0.252	16.0751	17.0857	-1.1204
17	14.9600	16.5804	0.252	16.0751	17.0857	-1.6204
18	20.1400	16.5804	0.252	16.0751	17.0857	3.5596
19	19.5100	16.5804	0.252	16.0751	17.0857	2.9296
20	16.3100	16.5804	0.252	16.0751	17.0857	-0.2704

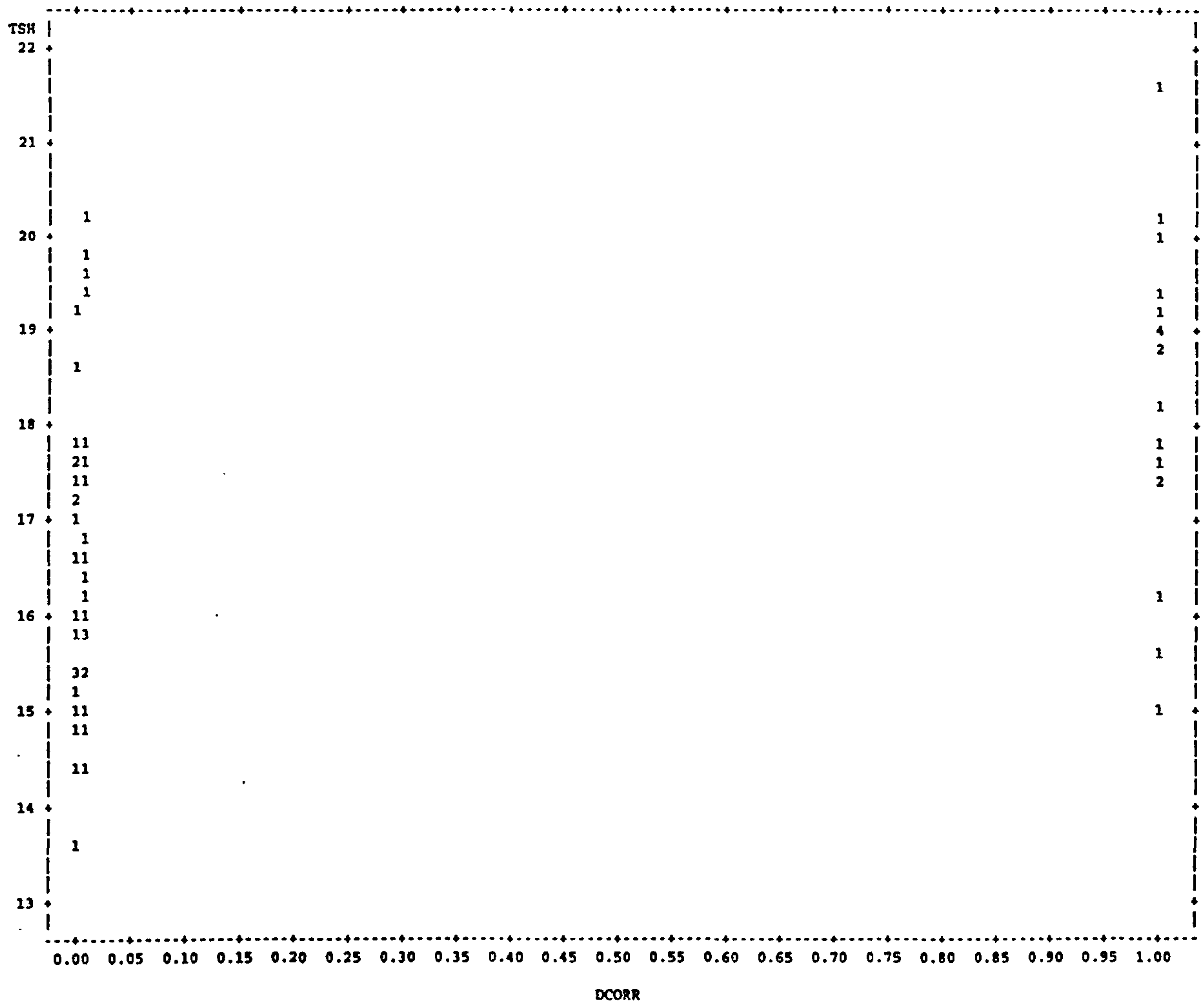
--- TMPT=92-95 ---

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
21	17.3100	18.3730	0.368	17.6361	19.1099	-1.0630
22	17.5000	18.3730	0.368	17.6361	19.1099	-0.8730
23	20.2700	18.3730	0.368	17.6361	19.1099	1.8970
24	18.9800	18.3730	0.368	17.6361	19.1099	0.6070
25	21.5200	18.3730	0.368	17.6361	19.1099	3.1470
26	20.0600	18.3730	0.368	17.6361	19.1099	1.6870
27	17.4200	18.3730	0.368	17.6361	19.1099	-0.9530
28	18.9600	18.3730	0.368	17.6361	19.1099	0.5870
29	18.9700	18.3730	0.368	17.6361	19.1099	0.5970
30	19.4100	18.3730	0.368	17.6361	19.1099	1.0370
31	17.3200	16.5623	0.255	16.0518	17.0727	0.7577
32	17.2700	16.5623	0.255	16.0518	17.0727	0.7077
33	16.9700	16.5623	0.255	16.0518	17.0727	0.4077
34	13.6500	16.5623	0.255	16.0518	17.0727	-2.9123
35	16.0600	16.5623	0.255	16.0518	17.0727	-0.5023
36	16.5100	16.5623	0.255	16.0518	17.0727	-0.0523
37	17.1500	16.5623	0.255	16.0518	17.0727	0.5877
38	15.3100	16.5623	0.255	16.0518	17.0727	-1.2523
39	19.1400	16.5623	0.255	16.0518	17.0727	2.5777
40	15.4100	16.5623	0.255	16.0518	17.0727	-1.1523
41	17.8500	16.5804	0.252	16.0751	17.0857	1.2696
42	15.7600	16.5804	0.252	16.0751	17.0857	-0.8204
43	17.4700	16.5804	0.252	16.0751	17.0857	0.8896
44	14.8000	16.5804	0.252	16.0751	17.0857	-1.7804
45	19.7600	16.5804	0.252	16.0751	17.0857	3.1796
46	19.4800	16.5804	0.252	16.0751	17.0857	2.8996

-- TMPT=92-95 --


T4
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00
DCORR

-- TMPT=92-95 --


TSH
22
21
20
19
18
17
16
15
14
13
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00
DCORR

45	F	15-18	5	18	3.45	122.78	12.33	n	0	-9.0	1.00
46	F	15-18	5	17	4.30	109.93	10.13	n	0	-9.0	1.00
47	F	15-18	5	16	4.46	121.80	12.02	n	0	-9.0	1.00
48	F	15-18	5	18	3.67	117.29	13.48	n	0	-9.0	1.00
49	F	15-18	5	18	3.81	127.88	11.70	n	0	-9.0	1.00
50	F	15-18	5	16	4.60	138.22	12.48	n	0	-9.0	1.00
51	F	15-18	5	15	4.61	108.50	13.14	n	0	-9.0	1.00
52	F	15-18	5	17	4.05	135.76	14.91	n	0	-9.0	1.00
53	F	15-18	5	15	4.07	129.06	12.63	n	0	-9.0	1.00
54	M	92-95	1	92	4.61	204.69	15.71	n	0	0.0	0.00
55	M	92-95	1	92	5.02	182.65	14.38	n	0	0.0	0.00
56	M	92-95	1	92	4.96	158.23	15.18	n	0	0.0	0.00

1 T4, T3, TSH data from Subchronic Perchlorate study 10:02 Monday, February 8, 1999 2

OBS	SEX	TMPT	DOSE	AGE	T4	T3	TSH	CODE	FOLL	INTDOSE	DCORR
57	M	92-95	1	93	4.63	156.89	17.72	n	0	0.0	0.00
58	M	92-95	1	93	6.35	189.84	17.50	n	0	0.0	0.00
59	M	92-95	1	94	5.19	179.28	15.39	n	0	0.0	0.00
60	M	92-95	1	94	5.12	215.33	17.61	ab	1	0.0	0.00
61	M	92-95	1	94	4.37	160.86	14.98	ab	1	0.0	0.00
62	M	92-95	1	95	4.97	171.09	14.79	n	0	0.0	0.00
63	M	92-95	1	95	5.38	179.51	18.61	n	0	0.0	0.00
64	M	92-95	2	92	3.22	158.15	16.27	n	0	5.1	0.01
65	M	92-95	2	92	4.22	155.40	16.87	n	0	5.1	0.01
66	M	92-95	2	92	4.70	136.19	15.95	n	0	5.1	0.01
67	M	92-95	2	93	4.23	152.61	17.54	n	0	5.1	0.01
68	M	92-95	2	93	4.12	154.06	15.40	n	0	5.1	0.01
69	M	92-95	2	94	4.76	144.06	15.46	n	0	5.1	0.01
70	M	92-95	2	94	4.38	186.86	14.96	n	0	5.1	0.01
71	M	92-95	2	94	4.49	149.51	20.14	n	0	5.1	0.01
72	M	92-95	2	95	4.65	151.61	19.51	n	0	5.1	0.01
73	M	92-95	2	95	4.79	186.19	16.31	n	0	5.1	0.01
74	M	92-95	5	92	3.87	134.53	17.31	n	0	-9.0	1.00
75	M	92-95	5	92	3.14	133.28	17.50	n	0	-9.0	1.00
76	M	92-95	5	92	3.84	115.98	20.27	n	0	-9.0	1.00
77	M	92-95	5	93	3.32	112.16	18.98	n	0	-9.0	1.00
78	M	92-95	5	93	3.59	103.33	21.52	n	0	-9.0	1.00
79	M	92-95	5	94	2.79	112.78	20.06	ab	1	-9.0	1.00
80	M	92-95	5	94	3.20	138.69	17.42	n	0	-9.0	1.00
81	M	92-95	5	94	3.61	129.19	18.96	n	0	-9.0	1.00
82	M	92-95	5	95	3.82	114.27	18.97	n	0	-9.0	1.00
83	M	92-95	5	95	3.49	123.71	19.41	n	0	-9.0	1.00
84	F	92-95	1	92	3.73	208.41	17.32	n	0	0.0	0.00
85	F	92-95	1	92	4.39	176.30	17.27	n	0	0.0	0.00
86	F	92-95	1	93	4.11	156.30	16.97	n	0	0.0	0.00
87	F	92-95	1	93	4.38	184.05	13.65	n	0	0.0	0.00
88	F	92-95	1	93	4.70	162.70	16.06	n	0	0.0	0.00
89	F	92-95	1	94	4.10	174.56	16.51	n	0	0.0	0.00

22	2	19534	4.19	3.43	92.3	-17.5	0.1
23	2	19536	4.62	.	.	-17.5	0.1
24	2	19542	4.94	3.30	77.9	-17.5	0.1
25	2	19562	4.71	.	.	-17.5	0.1
26	2	19575	4.20	3.23	90.9	-17.5	0.1
27	2	19576	4.09	3.05	83.3	-17.5	0.1
28	2	19590	4.30	3.84	74.5	-17.5	0.1
29	2	19593	4.76	3.03	94.9	-17.5	0.1
30	2	19594	4.89	.	.	-17.5	0.1
31	2	19605	5.25	3.67	86.1	-17.5	0.1
32	3	19504	4.56	3.69	74.3	-9.0	1.0
33	3	19521	4.74	3.50	89.5	-9.0	1.0
34	3	19535	4.04	2.92	91.3	-9.0	1.0
35	3	19538	5.13	2.91	75.5	-9.0	1.0
36	3	19539	4.40	3.15	80.9	-9.0	1.0
37	3	19547	4.51	3.11	78.1	-9.0	1.0
38	3	19568	4.39	3.31	.	-9.0	1.0
39	3	19579	4.38	.	.	-9.0	1.0
40	3	19584	4.89	2.71	70.8	-9.0	1.0
41	3	19586	4.22	.	.	-9.0	1.0
42	3	19587	5.61	2.98	78.9	-9.0	1.0
43	3	19588	4.98	.	.	-9.0	1.0
44	3	19589	5.63	.	.	-9.0	1.0
45	3	19592	4.78	3.18	71.0	-9.0	1.0
46	3	19596	5.28	2.84	83.5	-9.0	1.0
47	3	19597	4.03	.	.	-9.0	1.0
48	3	19603	4.88	3.33	.	-9.0	1.0
49	3	19616	5.11	3.15	.	-9.0	1.0
50	4	19522	4.34	.	.	3.0	3.0
51	4	19522	4.73	2.46	40.4	3.0	3.0
52	4	19525	5.01	2.28	38.9	3.0	3.0
53	4	19537	4.13	2.92	38.0	3.0	3.0
54	4	19544	5.58	2.73	46.1	3.0	3.0
55	4	19554	3.76	2.37	37.2	3.0	3.0

OBS	DOSE	LITTER	TSH	T4	T3	INTDOSE	DCORR
56	4	19557	5.03	2.52	42.4	3	3
57	4	19569	4.99	3.12	40.0	3	3
58	4	19573	3.97	.	.	3	3
59	4	19577	4.92	2.78	37.3	3	3
60	4	19598	4.60	2.76	34.5	3	3
61	4	19600	5.29	2.90	35.1	3	3
62	4	19604	4.95	.	43.4	3	3
63	4	19607	4.92	.	.	3	3
64	4	19612	4.95	.	.	3	3
65	4	19617	5.10	2.64	36.7	3	3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	1.16630	1.16630	7.700	0.0086
Error	37	5.60433	0.15147		
C Total	38	6.77064			

Root MSE	0.38919	R-square	0.1723
Dep Mean	3.10795	Adj R-sq	0.1499
C.V.	12.52239		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	2.981748	0.07715057	38.648	0.0001
INTDOSE	1	-0.021215	0.00764529	-2.775	0.0086

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	7141.94275	7141.94275	25.691	0.0001
Error	37	10285.95468	277.99878		
C Total	38	17427.89744			

Root MSE	16.67330	R-square	0.4098
Dep Mean	70.94872	Adj R-sq	0.3938
C.V.	23.50049		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	61.073094	3.30521267	18.478	0.0001
INTDOSE	1	-1.660126	0.32753252	-5.069	0.0001

Dependent Variable: TSH

23	.	3.3530	0.108	3.1340	3.5720	.
24	3.3000	3.3530	0.108	3.1340	3.5720	-0.0530
25	.	3.3530	0.108	3.1340	3.5720	.
26	3.2300	3.3530	0.108	3.1340	3.5720	-0.1230
27	3.0500	3.3530	0.108	3.1340	3.5720	-0.3030
28	3.8400	3.3530	0.108	3.1340	3.5720	0.4870
29	3.0300	3.3530	0.108	3.1340	3.5720	-0.3230
30	.	3.3530	0.108	3.1340	3.5720	.
31	3.6700	3.3530	0.108	3.1340	3.5720	0.3170
32	3.6900	3.1727	0.067	3.0379	3.3075	0.5173
33	3.5000	3.1727	0.067	3.0379	3.3075	0.3273
34	2.9200	3.1727	0.067	3.0379	3.3075	-0.2527
35	2.9100	3.1727	0.067	3.0379	3.3075	-0.2627
36	3.1500	3.1727	0.067	3.0379	3.3075	-0.0227
37	3.1100	3.1727	0.067	3.0379	3.3075	-0.0627
38	3.3100	3.1727	0.067	3.0379	3.3075	0.1373
39	.	3.1727	0.067	3.0379	3.3075	.
40	2.7100	3.1727	0.067	3.0379	3.3075	-0.4627
41	.	3.1727	0.067	3.0379	3.3075	.
42	2.9800	3.1727	0.067	3.0379	3.3075	-0.1927
43	.	3.1727	0.067	3.0379	3.3075	.
44	.	3.1727	0.067	3.0379	3.3075	.
45	3.1800	3.1727	0.067	3.0379	3.3075	0.00732
46	2.8400	3.1727	0.067	3.0379	3.3075	-0.3327
47	.	3.1727	0.067	3.0379	3.3075	.
48	3.3300	3.1727	0.067	3.0379	3.3075	0.1573
49	3.1500	3.1727	0.067	3.0379	3.3075	-0.0227
50	.	2.9181	0.093	2.7306	3.1056	.
51	2.4600	2.9181	0.093	2.7306	3.1056	-0.4581
52	2.2800	2.9181	0.093	2.7306	3.1056	-0.6381
53	2.9200	2.9181	0.093	2.7306	3.1056	0.00190
54	2.7300	2.9181	0.093	2.7306	3.1056	-0.1881

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 9

10:02 Monday, February 8, 1999

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
55	2.3700	2.9181	0.093	2.7306	3.1056	-0.5481
56	2.5200	2.9181	0.093	2.7306	3.1056	-0.3981
57	3.1200	2.9181	0.093	2.7306	3.1056	0.2019
58	.	2.9181	0.093	2.7306	3.1056	.
59	2.7800	2.9181	0.093	2.7306	3.1056	-0.1381
60	2.7600	2.9181	0.093	2.7306	3.1056	-0.1581
61	2.9000	2.9181	0.093	2.7306	3.1056	-0.0181
62	.	2.9181	0.093	2.7306	3.1056	.
63	.	2.9181	0.093	2.7306	3.1056	.
64	.	2.9181	0.093	2.7306	3.1056	.
65	2.6400	2.9181	0.093	2.7306	3.1056	-0.2781

34	91.3000	76.0142	2.851	70.2380	81.7905	15.2858
35	75.5000	76.0142	2.851	70.2380	81.7905	-0.5142
36	80.9000	76.0142	2.851	70.2380	81.7905	4.8858
37	78.1000	76.0142	2.851	70.2380	81.7905	2.0858
38	.	76.0142	2.851	70.2380	81.7905	.
39	.	76.0142	2.851	70.2380	81.7905	.
40	70.8000	76.0142	2.851	70.2380	81.7905	-5.2142
41	.	76.0142	2.851	70.2380	81.7905	.
42	78.9000	76.0142	2.851	70.2380	81.7905	2.8858
43	.	76.0142	2.851	70.2380	81.7905	.
44	.	76.0142	2.851	70.2380	81.7905	.
45	71.0000	76.0142	2.851	70.2380	81.7905	-5.0142
46	83.5000	76.0142	2.851	70.2380	81.7905	7.4858
47	.	76.0142	2.851	70.2380	81.7905	.
48	.	76.0142	2.851	70.2380	81.7905	.
49	.	76.0142	2.851	70.2380	81.7905	.
50	.	56.0927	3.965	48.0595	64.1260	.
51	40.4000	56.0927	3.965	48.0595	64.1260	-15.6927
52	38.9000	56.0927	3.965	48.0595	64.1260	-17.1927
53	38.0000	56.0927	3.965	48.0595	64.1260	-18.0927
54	46.1000	56.0927	3.965	48.0595	64.1260	-9.9927
55	37.2000	56.0927	3.965	48.0595	64.1260	-18.8927
56	42.4000	56.0927	3.965	48.0595	64.1260	-13.6927
57	40.0000	56.0927	3.965	48.0595	64.1260	-16.0927
58	.	56.0927	3.965	48.0595	64.1260	.
59	37.3000	56.0927	3.965	48.0595	64.1260	-18.7927
60	34.5000	56.0927	3.965	48.0595	64.1260	-21.5927
61	35.1000	56.0927	3.965	48.0595	64.1260	-20.9927
62	43.4000	56.0927	3.965	48.0595	64.1260	-12.6927
63	.	56.0927	3.965	48.0595	64.1260	.
64	.	56.0927	3.965	48.0595	64.1260	.
65	36.7000	56.0927	3.965	48.0595	64.1260	-19.3927
66	.	56.0927	3.965	48.0595	64.1260	.

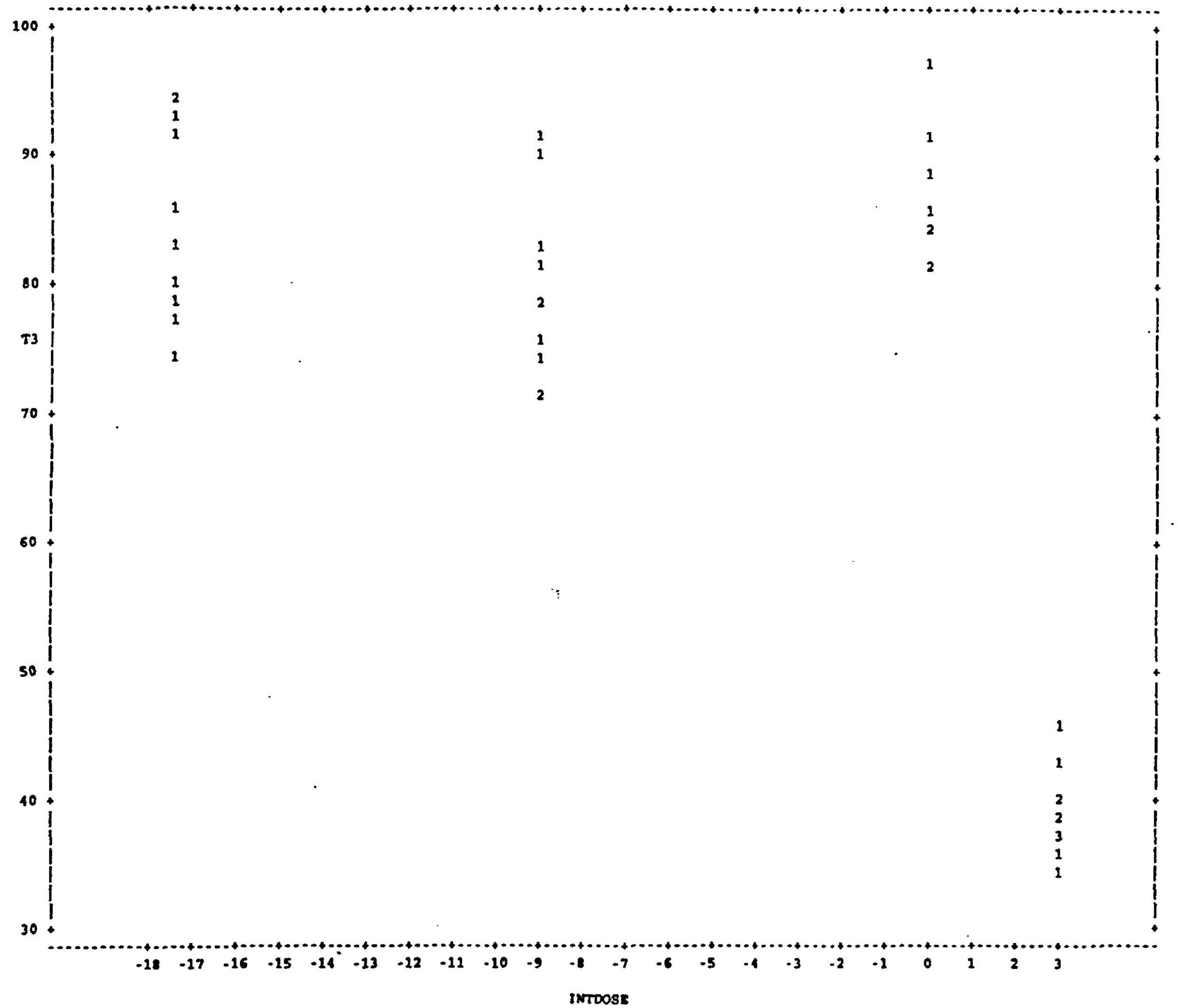
Sum of Residuals	0
Sum of Squared Residuals	10285.9547
Predicted Resid SS (Press)	11337.6685

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.1500	4.7424	0.095	4.5497	4.9350	-0.5924
2	4.2300	4.7424	0.095	4.5497	4.9350	-0.5124
3	4.3600	4.7424	0.095	4.5497	4.9350	-0.3824
4	4.0600	4.7424	0.095	4.5497	4.9350	-0.6824
5	4.1200	4.7424	0.095	4.5497	4.9350	-0.6224
6	4.6800	4.7424	0.095	4.5497	4.9350	-0.0624
7	4.1900	4.7424	0.095	4.5497	4.9350	-0.5524

51	4.7300	4.7702	0.114	4.5391	5.0013	-0.0402
52	5.0100	4.7702	0.114	4.5391	5.0013	0.2398
53	4.1300	4.7702	0.114	4.5391	5.0013	-0.6402
54	5.5800	4.7702	0.114	4.5391	5.0013	0.8098
55	3.7600	4.7702	0.114	4.5391	5.0013	-1.0102
56	5.0300	4.7702	0.114	4.5391	5.0013	0.2598
57	4.9900	4.7702	0.114	4.5391	5.0013	0.2198
58	3.9700	4.7702	0.114	4.5391	5.0013	-0.8002
59	4.9200	4.7702	0.114	4.5391	5.0013	0.1498
60	4.6000	4.7702	0.114	4.5391	5.0013	-0.1702
61	5.2900	4.7702	0.114	4.5391	5.0013	0.5198
62	4.9500	4.7702	0.114	4.5391	5.0013	0.1798
63	4.9200	4.7702	0.114	4.5391	5.0013	0.1498
64	4.9500	4.7702	0.114	4.5391	5.0013	0.1798
65	5.1000	4.7702	0.114	4.5391	5.0013	0.3298
66	4.8900	4.7702	0.114	4.5391	5.0013	0.1198

Sum of Residuals	0
Sum of Squared Residuals	8.5117
Predicted Resid SS (Press)	9.4559

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH APPROX. OF INTERNAL DOSE 14
10:02 Monday, February 8, 1999



Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	3.28404	3.28404	34.850	0.0001
Error	37	3.48660	0.09423		
C Total	38	6.77064			

Root MSE	0.30697	R-square	0.4850
Dep Mean	3.10795	Adj R-sq	0.4711
C.V.	9.87703		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	3.373094	0.06658424	50.659	0.0001
DCORR	1	-0.235015	0.03980993	-5.903	0.0001

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	15597.22790	15597.22790	315.238	0.0001
Error	37	1830.66953	49.47755		
C Total	38	17427.89744			

Root MSE	7.03403	R-square	0.8950
Dep Mean	70.94872	Adj R-sq	0.8921
C.V.	9.91424		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	89.221433	1.52572225	58.478	0.0001
DCORR	1	-16.196270	0.91221122	-17.755	0.0001

20	3.6700	3.3496	0.064	3.2200	3.4792	0.3204
21	2.8700	3.3496	0.064	3.2200	3.4792	-0.4796
22	3.4300	3.3496	0.064	3.2200	3.4792	0.0804
23	.	3.3496	0.064	3.2200	3.4792	.
24	3.3000	3.3496	0.064	3.2200	3.4792	-0.0496
25	.	3.3496	0.064	3.2200	3.4792	.
26	3.2300	3.3496	0.064	3.2200	3.4792	-0.1196
27	3.0500	3.3496	0.064	3.2200	3.4792	-0.2996
28	3.8400	3.3496	0.064	3.2200	3.4792	0.4904
29	3.0300	3.3496	0.064	3.2200	3.4792	-0.3196
30	.	3.3496	0.064	3.2200	3.4792	.
31	3.6700	3.3496	0.064	3.2200	3.4792	0.3204
32	3.6900	3.1381	0.049	3.0379	3.2382	0.5519
33	3.5000	3.1381	0.049	3.0379	3.2382	0.3619
34	2.9200	3.1381	0.049	3.0379	3.2382	-0.2181
35	2.9100	3.1381	0.049	3.0379	3.2382	-0.2281
36	3.1500	3.1381	0.049	3.0379	3.2382	0.0119
37	3.1100	3.1381	0.049	3.0379	3.2382	-0.0281
38	3.3100	3.1381	0.049	3.0379	3.2382	0.1719
39	.	3.1381	0.049	3.0379	3.2382	.
40	2.7100	3.1381	0.049	3.0379	3.2382	-0.4281
41	.	3.1381	0.049	3.0379	3.2382	.
42	2.9800	3.1381	0.049	3.0379	3.2382	-0.1581
43	.	3.1381	0.049	3.0379	3.2382	.
44	.	3.1381	0.049	3.0379	3.2382	.
45	3.1800	3.1381	0.049	3.0379	3.2382	0.0419
46	2.8400	3.1381	0.049	3.0379	3.2382	-0.2981
47	.	3.1381	0.049	3.0379	3.2382	.
48	3.3300	3.1381	0.049	3.0379	3.2382	0.1919
49	3.1500	3.1381	0.049	3.0379	3.2382	0.0119
50	.	2.6680	0.089	2.4872	2.8489	.
51	2.4600	2.6680	0.089	2.4872	2.8489	-0.2080
52	2.2800	2.6680	0.089	2.4872	2.8489	-0.3880
53	2.9200	2.6680	0.089	2.4872	2.8489	0.2520
54	2.7300	2.6680	0.089	2.4872	2.8489	0.0620
55	2.3700	2.6680	0.089	2.4872	2.8489	-0.2980

1 DEV NT STUDY, PND5 PUPS, CORRELATIONS WITH EXTERNAL DOSE 10:02 Monday, February 8, 1999 19

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
56	2.5200	2.6680	0.089	2.4872	2.8489	-0.1480
57	3.1200	2.6680	0.089	2.4872	2.8489	0.4520
58	.	2.6680	0.089	2.4872	2.8489	.
59	2.7800	2.6680	0.089	2.4872	2.8489	0.1120
60	2.7600	2.6680	0.089	2.4872	2.8489	0.0920
61	2.9000	2.6680	0.089	2.4872	2.8489	0.2320
62	.	2.6680	0.089	2.4872	2.8489	.
63	.	2.6680	0.089	2.4872	2.8489	.

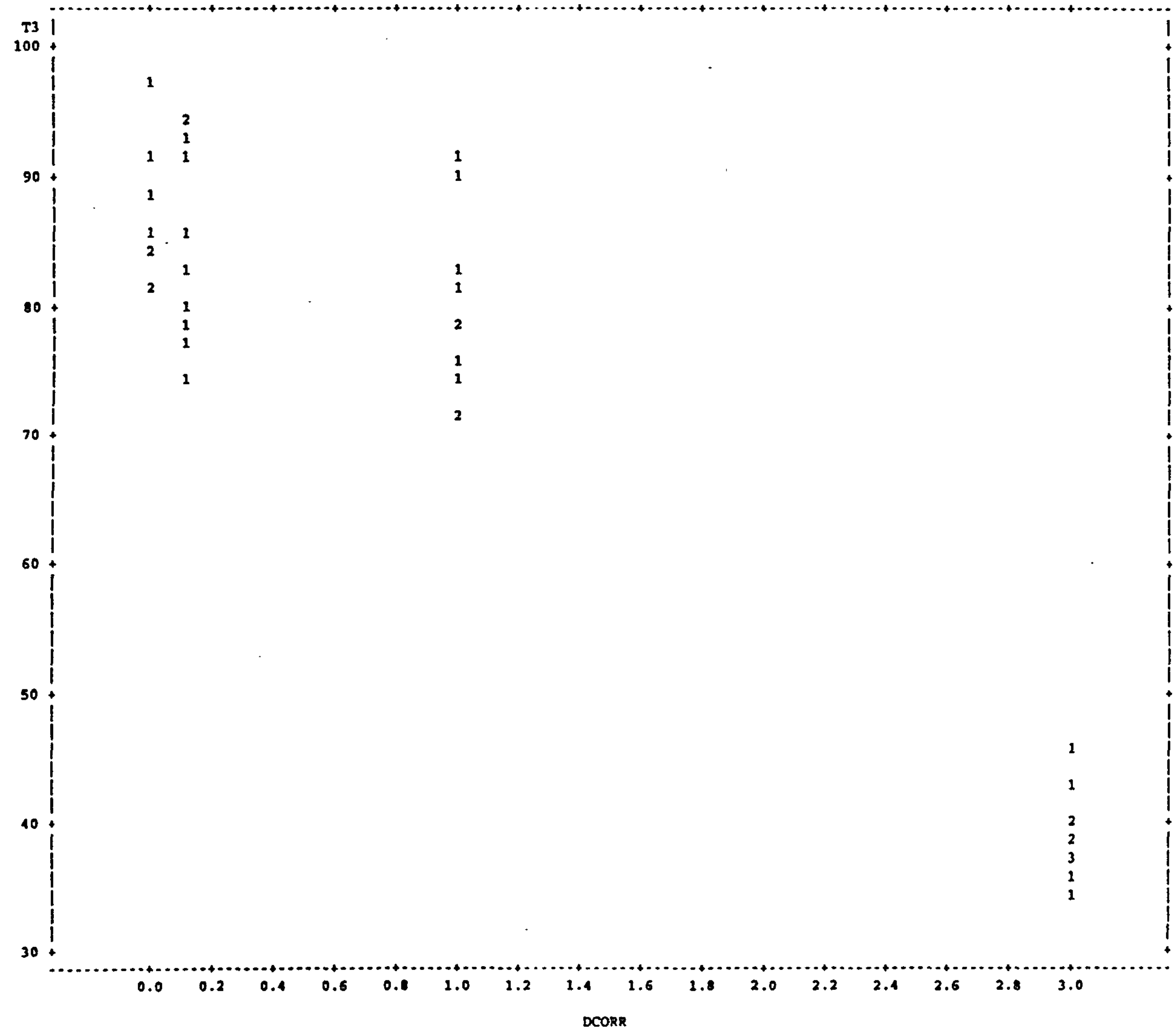
Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
36	80.9000	73.0252	1.132	70.7307	75.3196	7.8748
37	78.1000	73.0252	1.132	70.7307	75.3196	5.0748
38	.	73.0252	1.132	70.7307	75.3196	.
39	.	73.0252	1.132	70.7307	75.3196	.
40	70.8000	73.0252	1.132	70.7307	75.3196	-2.2252
41	.	73.0252	1.132	70.7307	75.3196	.
42	78.9000	73.0252	1.132	70.7307	75.3196	5.8748
43	.	73.0252	1.132	70.7307	75.3196	.
44	.	73.0252	1.132	70.7307	75.3196	.
45	71.0000	73.0252	1.132	70.7307	75.3196	-2.0252
46	83.5000	73.0252	1.132	70.7307	75.3196	10.4748
47	.	73.0252	1.132	70.7307	75.3196	.
48	.	73.0252	1.132	70.7307	75.3196	.
49	.	73.0252	1.132	70.7307	75.3196	.
50	.	40.6326	2.046	36.4880	44.7772	.
51	40.4000	40.6326	2.046	36.4880	44.7772	-0.2326
52	38.9000	40.6326	2.046	36.4880	44.7772	-1.7326
53	38.0000	40.6326	2.046	36.4880	44.7772	-2.6326
54	46.1000	40.6326	2.046	36.4880	44.7772	5.4674
55	37.2000	40.6326	2.046	36.4880	44.7772	-3.4326
56	42.4000	40.6326	2.046	36.4880	44.7772	1.7674
57	40.0000	40.6326	2.046	36.4880	44.7772	-0.6326
58	.	40.6326	2.046	36.4880	44.7772	.
59	37.3000	40.6326	2.046	36.4880	44.7772	-3.3326
60	34.5000	40.6326	2.046	36.4880	44.7772	-6.1326
61	35.1000	40.6326	2.046	36.4880	44.7772	-5.5326
62	43.4000	40.6326	2.046	36.4880	44.7772	2.7674
63	.	40.6326	2.046	36.4880	44.7772	.
64	.	40.6326	2.046	36.4880	44.7772	.
65	36.7000	40.6326	2.046	36.4880	44.7772	-3.9326
66	.	40.6326	2.046	36.4880	44.7772	.

Sum of Residuals	0
Sum of Squared Residuals	1830.6695
Predicted Resid SS (Press)	1984.5367

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.1500	4.5800	0.102	4.3732	4.7869	-0.4300
2	4.2300	4.5800	0.102	4.3732	4.7869	-0.3500
3	4.3600	4.5800	0.102	4.3732	4.7869	-0.2200
4	4.0600	4.5800	0.102	4.3732	4.7869	-0.5200
5	4.1200	4.5800	0.102	4.3732	4.7869	-0.4600
6	4.6800	4.5800	0.102	4.3732	4.7869	0.1000

51	4.7300	4.8649	0.137	4.5876	5.1423	-0.1349
52	5.0100	4.8649	0.137	4.5876	5.1423	0.1451
53	4.1300	4.8649	0.137	4.5876	5.1423	-0.7349
54	5.5800	4.8649	0.137	4.5876	5.1423	0.7151
55	3.7600	4.8649	0.137	4.5876	5.1423	-1.1049
56	5.0300	4.8649	0.137	4.5876	5.1423	0.1651
57	4.9900	4.8649	0.137	4.5876	5.1423	0.1251
58	3.9700	4.8649	0.137	4.5876	5.1423	-0.8949
59	4.9200	4.8649	0.137	4.5876	5.1423	0.0551
60	4.6000	4.8649	0.137	4.5876	5.1423	-0.2649
61	5.2900	4.8649	0.137	4.5876	5.1423	0.4251
62	4.9500	4.8649	0.137	4.5876	5.1423	0.0851
63	4.9200	4.8649	0.137	4.5876	5.1423	0.0551
64	4.9500	4.8649	0.137	4.5876	5.1423	0.0851
65	5.1000	4.8649	0.137	4.5876	5.1423	0.2351
66	4.8900	4.8649	0.137	4.5876	5.1423	0.0251

Sum of Residuals	0
Sum of Squared Residuals	8.1984
Predicted Resid SS (Press)	9.1615



T3
100
90
80
70
60
50
40
30
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0
DCORR

--- TMPT=15-18 ---

Correlation Analysis

5 'VAR' Variables: DCORR INTDOSE T4 T3 TSH

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DCORR	53	0.380189	0.487156	20.150000	0	1.000000
INTDOSE	53	-1.952830	5.896596	-103.500000	-9.000000	5.100000
T4	53	4.742075	0.697466	251.330000	3.240000	6.640000
T3	53	142.397547	28.942102	7547.070000	101.610000	234.210000
TSH	53	13.864906	3.271930	734.840000	9.000000	21.750000

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 53

	DCORR	INTDOSE	T4	T3	TSH
DCORR	1.00000	-0.93648	-0.10798	-0.48964	0.44024
	0.0	0.0001	0.4415	0.0002	0.0010
INTDOSE	-0.93648	1.00000	0.04070	0.37723	-0.38054
	0.0001	0.0	0.7723	0.0054	0.0049
T4	-0.10798	0.04070	1.00000	0.38512	0.52428
	0.4415	0.7723	0.0	0.0044	0.0001
T3	-0.48964	0.37723	0.38512	1.00000	0.08258
	0.0002	0.0054	0.0044	0.0	0.5566
TSH	0.44024	-0.38054	0.52428	0.08258	1.00000
	0.0010	0.0049	0.0001	0.5566	0.0

--- TMPT=92-95 ---

Correlation Analysis

5 'VAR' Variables: DCORR INTDOSE T4 T3 TSH

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.751477	0.10191847	46.620	0.0001
INTDOSE	1	0.004815	0.01654918	0.291	0.7723

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	6198.44222	6198.44222	8.462	0.0054
Error	51	37359.11316	732.53163		
C Total	52	43557.55538			

Root MSE	27.06532	R-square	0.1423
Dep Mean	142.39755	Adj R-sq	0.1255
C.V.	19.00687		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	146.013333	3.92000322	37.248	0.0001
INTDOSE	1	1.851562	0.63651717	2.909	0.0054

--- TMPT=15-18 ---

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	80.61296	80.61296	8.636	0.0049
Error	51	476.07436	9.33479		
C Total	52	556.68732			

Root MSE	3.05529	R-square	0.1448

31	4.3600	4.7515	0.102	4.5469	4.9561	-0.3915
32	4.7500	4.7515	0.102	4.5469	4.9561	-0.00148
33	4.7200	4.7515	0.102	4.5469	4.9561	-0.0315
34	4.4800	4.7760	0.152	4.4718	5.0803	-0.2960
35	4.3900	4.7760	0.152	4.4718	5.0803	-0.3860
36	4.5900	4.7760	0.152	4.4718	5.0803	-0.1860
37	4.5800	4.7760	0.152	4.4718	5.0803	-0.1960
38	4.7400	4.7760	0.152	4.4718	5.0803	-0.0360
39	4.6000	4.7760	0.152	4.4718	5.0803	-0.1760
40	3.2400	4.7760	0.152	4.4718	5.0803	-1.5360
41	4.4400	4.7760	0.152	4.4718	5.0803	-0.3360
42	3.8300	4.7760	0.152	4.4718	5.0803	-0.9460
43	4.3400	4.7760	0.152	4.4718	5.0803	-0.4360
44	4.0200	4.7081	0.151	4.4041	5.0122	-0.6881
45	3.4500	4.7081	0.151	4.4041	5.0122	-1.2581
46	4.3000	4.7081	0.151	4.4041	5.0122	-0.4081
47	4.4600	4.7081	0.151	4.4041	5.0122	-0.2481
48	3.6700	4.7081	0.151	4.4041	5.0122	-1.0381
49	3.8100	4.7081	0.151	4.4041	5.0122	-0.8981
50	4.6000	4.7081	0.151	4.4041	5.0122	-0.1081
51	4.6100	4.7081	0.151	4.4041	5.0122	-0.0981
52	4.0500	4.7081	0.151	4.4041	5.0122	-0.6581

-- TMPT=15-18 --

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
53	4.0700	4.7081	0.151	4.4041	5.0122	-0.6381

Sum of Residuals	0
Sum of Squared Residuals	25.2540
Predicted Resid SS (Press)	27.2588

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	200.1	146.0	3.920	138.1	153.9	54.1267
2	208.5	146.0	3.920	138.1	153.9	62.4367
3	186.2	146.0	3.920	138.1	153.9	40.2167
4	234.2	146.0	3.920	138.1	153.9	88.1967
5	205.1	146.0	3.920	138.1	153.9	59.0667
6	178.5	146.0	3.920	138.1	153.9	32.5167
7	170.2	146.0	3.920	138.1	153.9	24.1467
8	187.1	146.0	3.920	138.1	153.9	41.0967
9	173.2	155.5	5.829	143.8	167.2	17.7737

51	108.5	129.3	5.826	117.7	141.0	-20.8493
52	135.8	129.3	5.826	117.7	141.0	6.4107
53	129.1	129.3	5.826	117.7	141.0	-0.2893

Sum of Residuals	0
Sum of Squared Residuals	37359.1132
Predicted Resid SS (Press)	39573.3004

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	13.0800	13.4526	0.443	12.5642	14.3409	-0.3726
2	13.7200	13.4526	0.443	12.5642	14.3409	0.2674
3	14.2000	13.4526	0.443	12.5642	14.3409	0.7474
4	16.3800	13.4526	0.443	12.5642	14.3409	2.9274
5	13.2100	13.4526	0.443	12.5642	14.3409	-0.2426
6	15.5200	13.4526	0.443	12.5642	14.3409	2.0674
7	17.4500	13.4526	0.443	12.5642	14.3409	3.9974
8	15.3000	13.4526	0.443	12.5642	14.3409	1.8474
9	15.2200	12.3757	0.658	11.0547	13.6966	2.8443
10	12.7600	12.3757	0.658	11.0547	13.6966	0.3843
11	17.6300	12.3757	0.658	11.0547	13.6966	5.2543
12	18.4100	12.3757	0.658	11.0547	13.6966	6.0343
13	16.4900	12.3757	0.658	11.0547	13.6966	4.1143
14	19.9300	15.3529	0.658	14.0326	16.6733	4.5771
15	20.2700	15.3529	0.658	14.0326	16.6733	4.9171
16	17.3900	15.3529	0.658	14.0326	16.6733	2.0371
17	17.0300	15.3529	0.658	14.0326	16.6733	1.6771
18	20.8700	15.3529	0.658	14.0326	16.6733	5.5171
19	18.7200	15.3529	0.658	14.0326	16.6733	3.3671
20	21.7500	15.3529	0.658	14.0326	16.6733	6.3971
21	16.1100	15.3529	0.658	14.0326	16.6733	0.7571
22	17.4800	15.3529	0.658	14.0326	16.6733	2.1271
23	18.4500	15.3529	0.658	14.0326	16.6733	3.0971
24	9.8900	13.4526	0.443	12.5642	14.3409	-3.5626
25	9.4900	13.4526	0.443	12.5642	14.3409	-3.9626
26	9.0000	13.4526	0.443	12.5642	14.3409	-4.4526
27	10.5100	13.4526	0.443	12.5642	14.3409	-2.9426
28	10.8600	13.4526	0.443	12.5642	14.3409	-2.5926
29	11.3000	13.4526	0.443	12.5642	14.3409	-2.1526
30	10.2700	13.4526	0.443	12.5642	14.3409	-3.1826
31	11.1500	13.4526	0.443	12.5642	14.3409	-2.3026
32	10.5700	13.4526	0.443	12.5642	14.3409	-2.8826

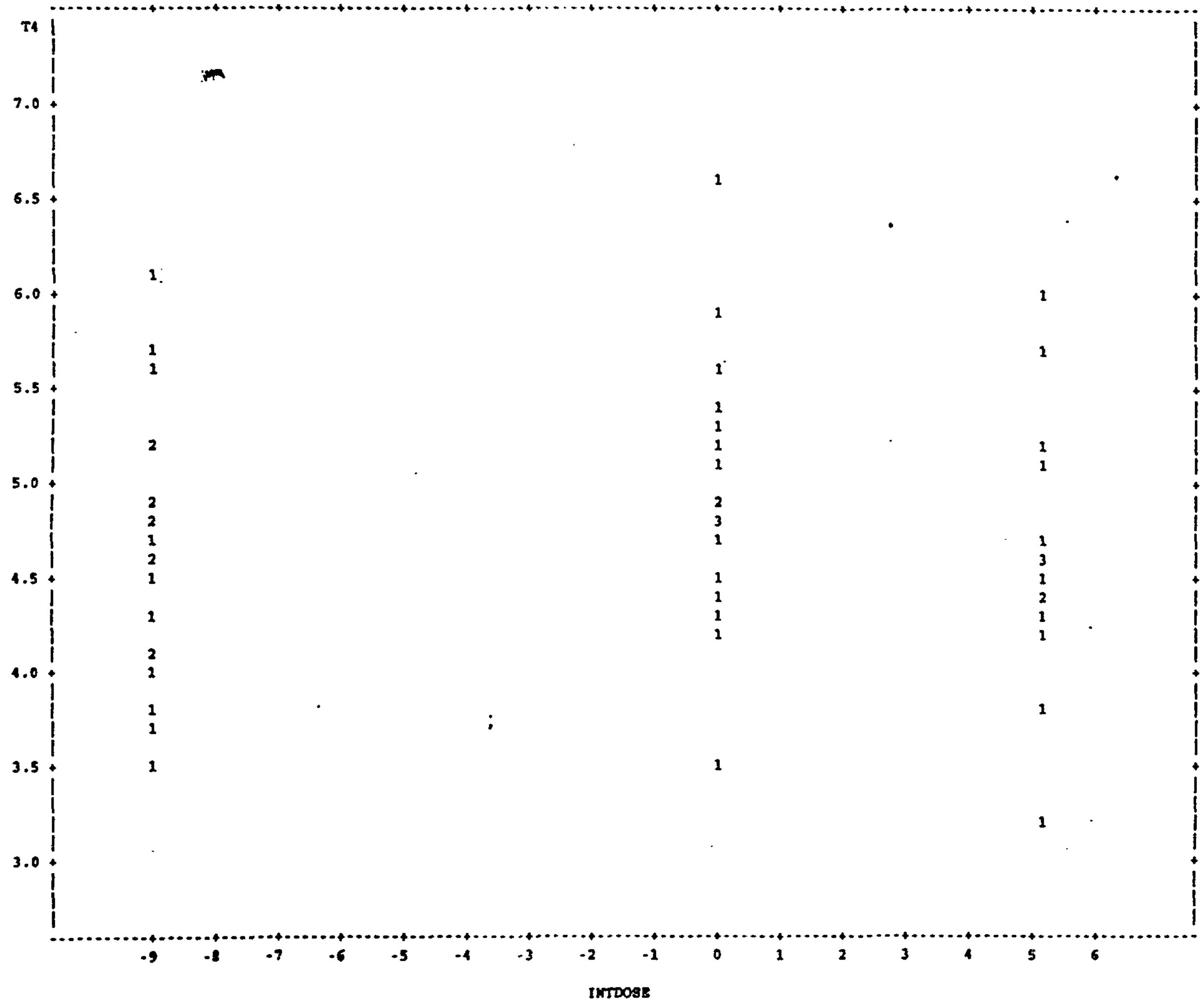
1 Subchronic Rat Perchlorate Study, Correlations with int. dose 32

10:02 Monday, February 8, 1999

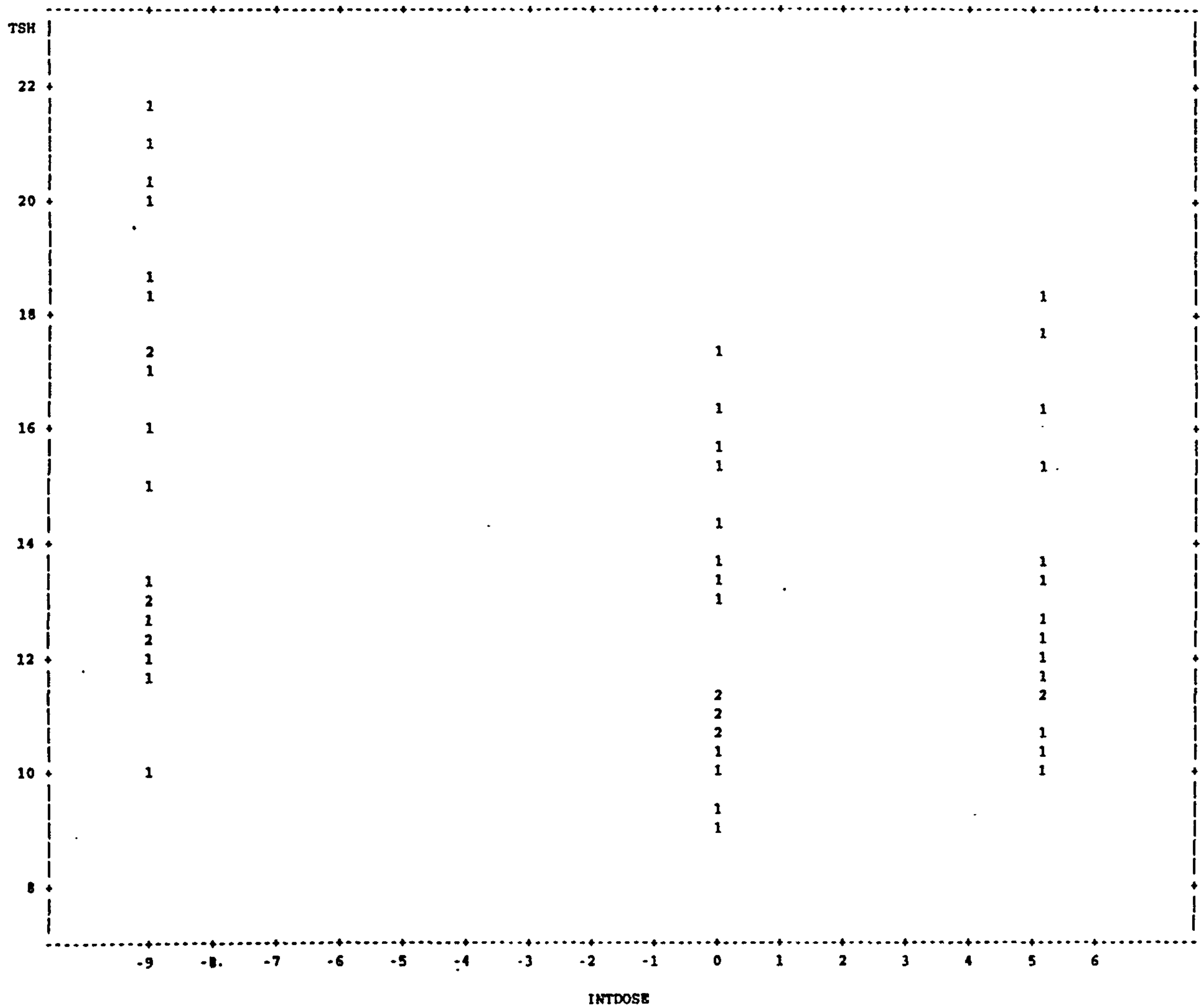
---------- TMPT=15-18 ----------

1 Subchronic Rat Perchlorate Study, Correlations with int. dose 33
10:02 Monday, February 8, 1999

-- TMPT=15-18 --



THPT=15-18



INTERCEP	1	152.405922	3.04588710	50.037	0.0001
INTDOSE	1	2.409122	0.51557345	4.673	0.0001

---------- TMPT=92-95 ----------

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	29.86510	29.86510	10.732	0.0018
Error	57	158.61706	2.78276		
C Total	58	188.48216			

Root MSE	1.66816	R-square	0.1585
Dep Mean	17.15153	Adj R-sq	0.1437
C.V.	9.72601		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	17.007841	0.22156043	76.764	0.0001
INTDOSE	1	-0.122861	0.03750325	-3.276	0.0018

---------- TMPT=92-95 ----------

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	4.6100	4.0832	0.094	3.8941	4.2722	0.5268
2	5.0200	4.0832	0.094	3.8941	4.2722	0.9368
3	4.9600	4.0832	0.094	3.8941	4.2722	0.8768
4	4.6300	4.0832	0.094	3.8941	4.2722	0.5468
5	6.3500	4.0832	0.094	3.8941	4.2722	2.2668
6	5.1900	4.0832	0.094	3.8941	4.2722	1.1068
7	5.1200	4.0832	0.094	3.8941	4.2722	1.0368
8	4.3700	4.0832	0.094	3.8941	4.2722	0.2868
9	4.9700	4.0832	0.094	3.8941	4.2722	0.8868
10	5.3800	4.0832	0.094	3.8941	4.2722	1.2968

53	3.4100	3.5867	0.156	3.2751	3.8983	-0.1767
54	2.9000	3.5867	0.156	3.2751	3.8983	-0.6867
55	3.7800	3.5867	0.156	3.2751	3.8983	0.1933
56	3.4300	3.5867	0.156	3.2751	3.8983	-0.1567
57	3.1800	3.5867	0.156	3.2751	3.8983	-0.4067
58	2.7900	3.5867	0.156	3.2751	3.8983	-0.7967
59	3.0400	3.5867	0.156	3.2751	3.8983	-0.5467

Sum of Residuals	0
Sum of Squared Residuals	28.7923
Predicted Resid SS (Press)	30.4899

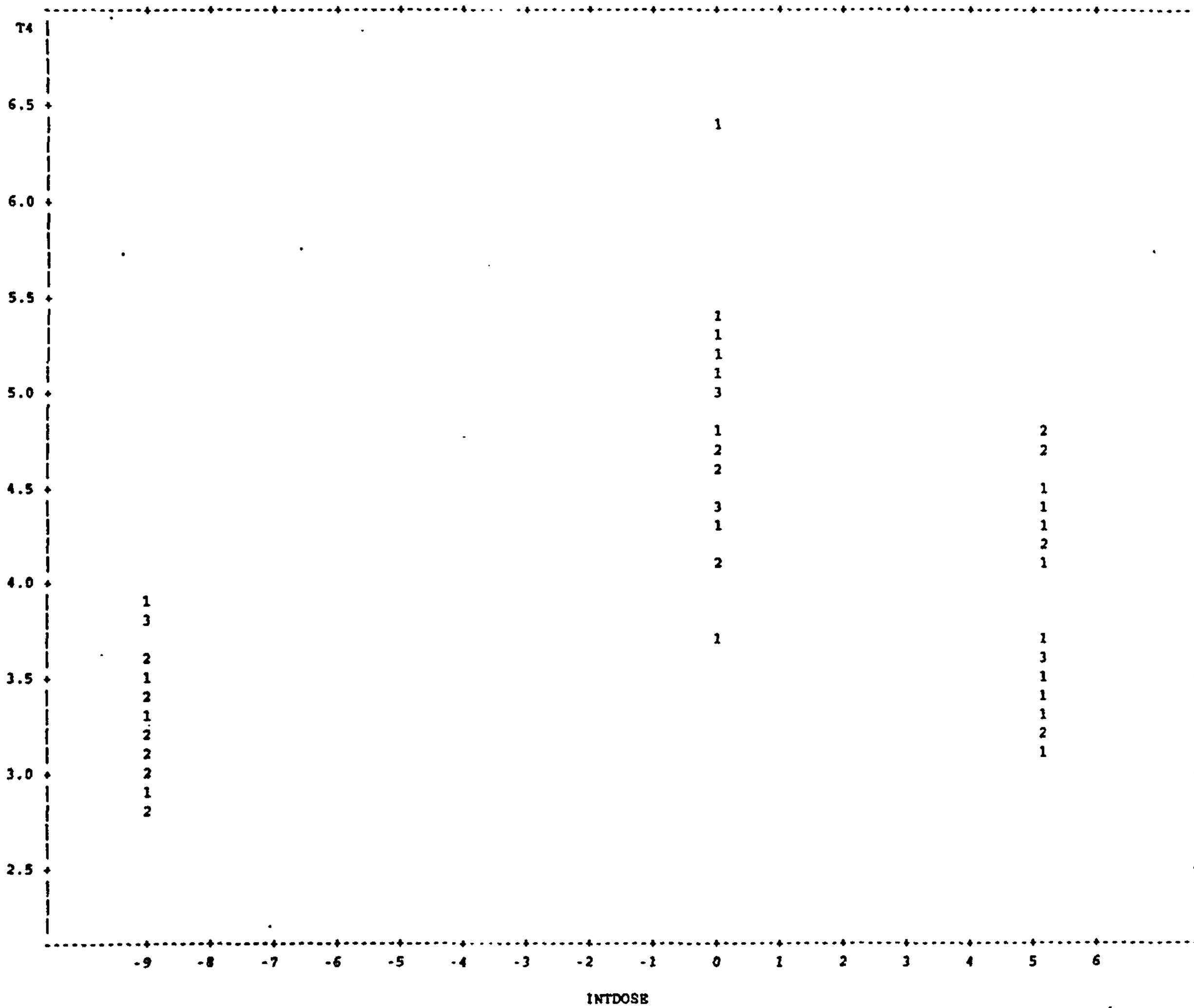
Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	204.7	152.4	3.046	146.3	158.5	52.2841
2	182.7	152.4	3.046	146.3	158.5	30.2441
3	158.2	152.4	3.046	146.3	158.5	5.8241
4	156.9	152.4	3.046	146.3	158.5	4.4841
5	189.8	152.4	3.046	146.3	158.5	37.4341
6	179.3	152.4	3.046	146.3	158.5	26.8741
7	215.3	152.4	3.046	146.3	158.5	62.9241
8	160.9	152.4	3.046	146.3	158.5	8.4541
9	171.1	152.4	3.046	146.3	158.5	18.6841
10	179.5	152.4	3.046	146.3	158.5	27.1041
11	158.2	164.7	4.400	155.9	173.5	-6.5424
12	155.4	164.7	4.400	155.9	173.5	-9.2924
13	136.2	164.7	4.400	155.9	173.5	-28.5024
14	152.6	164.7	4.400	155.9	173.5	-12.0824
15	154.1	164.7	4.400	155.9	173.5	-10.6324
16	144.1	164.7	4.400	155.9	173.5	-20.6324
17	186.9	164.7	4.400	155.9	173.5	22.1676
18	149.5	164.7	4.400	155.9	173.5	-15.1824
19	151.6	164.7	4.400	155.9	173.5	-13.0824
20	186.2	164.7	4.400	155.9	173.5	21.4976
21	134.5	130.7	5.021	120.7	140.8	3.8062
22	133.3	130.7	5.021	120.7	140.8	2.5562
23	116.0	130.7	5.021	120.7	140.8	-14.7438
24	112.2	130.7	5.021	120.7	140.8	-18.5638
25	103.3	130.7	5.021	120.7	140.8	-27.3938
26	112.8	130.7	5.021	120.7	140.8	-17.9438
27	138.7	130.7	5.021	120.7	140.8	7.9662
28	129.2	130.7	5.021	120.7	140.8	-1.5338
29	114.3	130.7	5.021	120.7	140.8	-16.4538
30	123.7	130.7	5.021	120.7	140.8	-7.0138
31	208.4	152.4	3.046	146.3	158.5	56.0041
32	176.3	152.4	3.046	146.3	158.5	23.8941

6	15.3900	17.0078	0.222	16.5642	17.4515	-1.6178
7	17.6100	17.0078	0.222	16.5642	17.4515	0.6022
8	14.9800	17.0078	0.222	16.5642	17.4515	-2.0278
9	14.7900	17.0078	0.222	16.5642	17.4515	-2.2178
10	18.6100	17.0078	0.222	16.5642	17.4515	1.6022
11	16.2700	16.3813	0.320	15.7403	17.0222	-0.1113
12	16.8700	16.3813	0.320	15.7403	17.0222	0.4887
13	15.9500	16.3813	0.320	15.7403	17.0222	-0.4313
14	17.5400	16.3813	0.320	15.7403	17.0222	1.1587
15	15.4000	16.3813	0.320	15.7403	17.0222	-0.9813
16	15.4600	16.3813	0.320	15.7403	17.0222	-0.9213
17	14.9600	16.3813	0.320	15.7403	17.0222	-1.4213
18	20.1400	16.3813	0.320	15.7403	17.0222	3.7587
19	19.5100	16.3813	0.320	15.7403	17.0222	3.1287
20	16.3100	16.3813	0.320	15.7403	17.0222	-0.0713

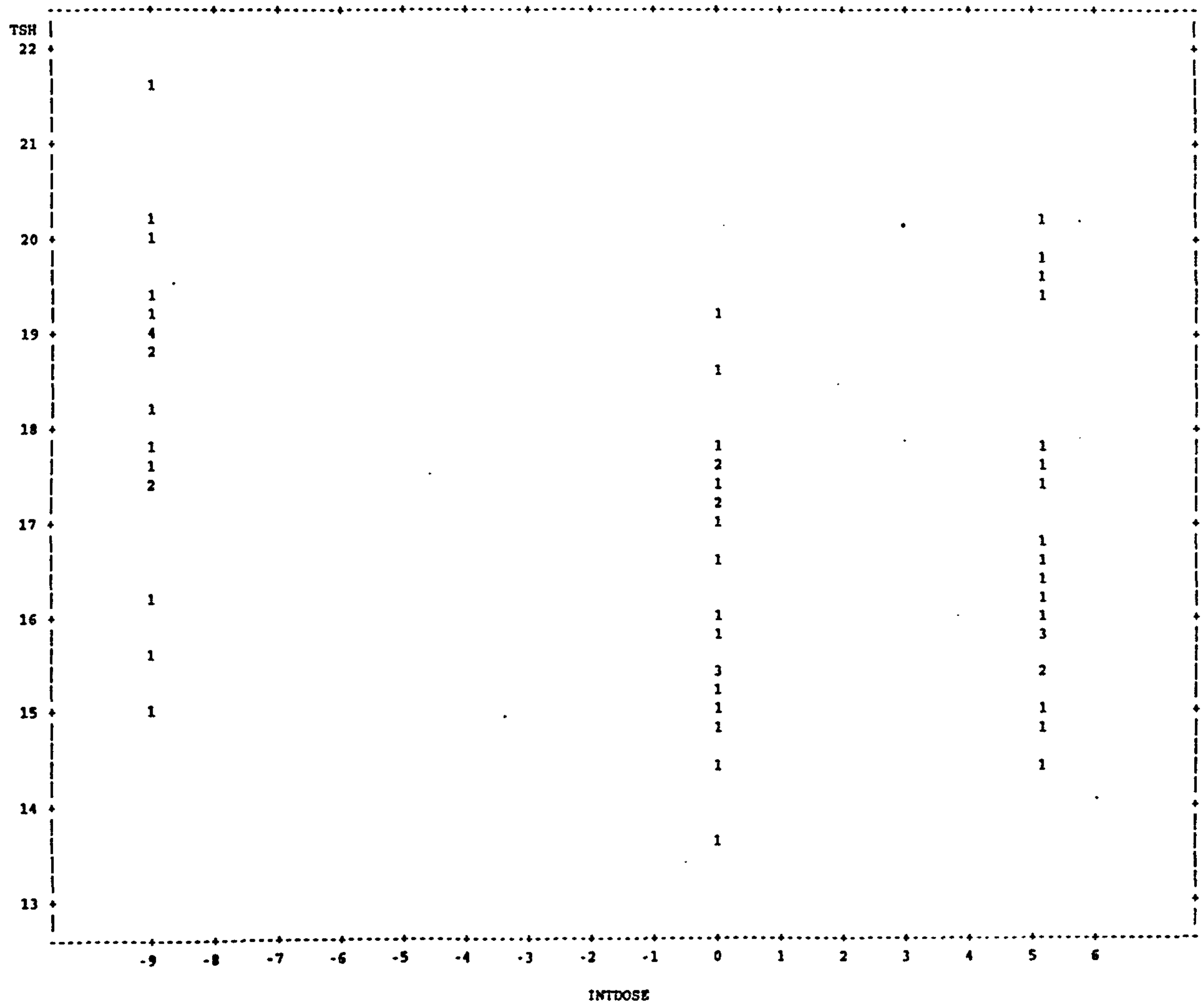
-- TMPT=92-95 --

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
21	17.3100	18.1136	0.365	17.3822	18.8450	-0.8036
22	17.5000	18.1136	0.365	17.3822	18.8450	-0.6136
23	20.2700	18.1136	0.365	17.3822	18.8450	2.1564
24	18.9800	18.1136	0.365	17.3822	18.8450	0.8664
25	21.5200	18.1136	0.365	17.3822	18.8450	3.4064
26	20.0600	18.1136	0.365	17.3822	18.8450	1.9464
27	17.4200	18.1136	0.365	17.3822	18.8450	-0.6936
28	18.9600	18.1136	0.365	17.3822	18.8450	0.8464
29	18.9700	18.1136	0.365	17.3822	18.8450	0.8564
30	19.4100	18.1136	0.365	17.3822	18.8450	1.2964
31	17.3200	17.0078	0.222	16.5642	17.4515	0.3122
32	17.2700	17.0078	0.222	16.5642	17.4515	0.2622
33	16.9700	17.0078	0.222	16.5642	17.4515	-0.0378
34	13.6500	17.0078	0.222	16.5642	17.4515	-3.3578
35	16.0600	17.0078	0.222	16.5642	17.4515	-0.9478
36	16.5100	17.0078	0.222	16.5642	17.4515	-0.4978
37	17.1500	17.0078	0.222	16.5642	17.4515	0.1422
38	15.3100	17.0078	0.222	16.5642	17.4515	-1.6978
39	19.1400	17.0078	0.222	16.5642	17.4515	2.1322
40	15.4100	17.0078	0.222	16.5642	17.4515	-1.5978
41	17.8500	16.3813	0.320	15.7403	17.0222	1.4687
42	15.7600	16.3813	0.320	15.7403	17.0222	-0.6213
43	17.4700	16.3813	0.320	15.7403	17.0222	1.0887
44	14.8000	16.3813	0.320	15.7403	17.0222	-1.5813
45	19.7600	16.3813	0.320	15.7403	17.0222	3.3787
46	19.4800	16.3813	0.320	15.7403	17.0222	3.0987

-- TMPT=92-95 --



-- TMPT=92-95 --



INTERCEP	1	153.457081	4.45604412	34.438	0.0001
DCORR	1	-29.089592	7.25364227	-4.010	0.0002

1 Subchronic Rat Perchlorate Study, Correlations with external dose 46

10:02 Monday, February 8, 1999

-- TMPT=15-18 --

Dependent Variable: TSH

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	107.89105	107.89105	12.260	0.0010
Error	51	448.79628	8.79993		
C Total	52	556.68732			

Root MSE	2.96647	R-square	0.1938
Dep Mean	13.86491	Adj R-sq	0.1780
C.V.	21.39551		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	12.740761	0.51875586	24.560	0.0001
DCORR	1	2.956806	0.84444170	3.501	0.0010

1 Subchronic Rat Perchlorate Study, Correlations with external dose 47

10:02 Monday, February 8, 1999

-- TMPT=15-18 --

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	5.1000	4.8009	0.122	4.5550	5.0467	0.2991
2	5.6400	4.8009	0.122	4.5550	5.0467	0.8391
3	6.6400	4.8009	0.122	4.5550	5.0467	1.8391
4	5.8800	4.8009	0.122	4.5550	5.0467	1.0791
5	4.8400	4.8009	0.122	4.5550	5.0467	0.0391
6	5.4200	4.8009	0.122	4.5550	5.0467	0.6191
7	5.2900	4.8009	0.122	4.5550	5.0467	0.4891
8	5.1900	4.8009	0.122	4.5550	5.0467	0.3891
9	5.1000	4.7993	0.121	4.5560	5.0427	0.3007
10	4.2300	4.7993	0.121	4.5560	5.0427	-0.5693

53	4.0700	4.6463	0.157	4.3320	4.9606	-0.5763

Sum of Residuals 0
Sum of Squared Residuals 25.0009
Predicted Resid SS (Press) 26.9981

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	200.1	153.5	4.456	144.5	162.4	46.6829
2	208.5	153.5	4.456	144.5	162.4	54.9929
3	186.2	153.5	4.456	144.5	162.4	32.7729
4	234.2	153.5	4.456	144.5	162.4	80.7529
5	205.1	153.5	4.456	144.5	162.4	51.6229
6	178.5	153.5	4.456	144.5	162.4	25.0729
7	170.2	153.5	4.456	144.5	162.4	16.7029
8	187.1	153.5	4.456	144.5	162.4	33.6529
9	173.2	153.2	4.412	144.3	162.0	20.0638
10	170.5	153.2	4.412	144.3	162.0	17.3238
11	154.8	153.2	4.412	144.3	162.0	1.6438
12	136.3	153.2	4.412	144.3	162.0	-16.8662
13	183.3	153.2	4.412	144.3	162.0	30.1338
14	122.4	124.4	5.698	112.9	135.8	-1.9775
15	130.7	124.4	5.698	112.9	135.8	6.3425
16	139.6	124.4	5.698	112.9	135.8	15.1925
17	137.4	124.4	5.698	112.9	135.8	13.0725
18	133.2	124.4	5.698	112.9	135.8	8.8125
19	101.6	124.4	5.698	112.9	135.8	-22.7575
20	125.9	124.4	5.698	112.9	135.8	1.5425
21	109.9	124.4	5.698	112.9	135.8	-14.4375
22	124.6	124.4	5.698	112.9	135.8	0.2025
23	112.3	124.4	5.698	112.9	135.8	-12.0975
24	133.3	153.5	4.456	144.5	162.4	-20.1771
25	147.0	153.5	4.456	144.5	162.4	-6.4771
26	148.5	153.5	4.456	144.5	162.4	-4.9371
27	113.0	153.5	4.456	144.5	162.4	-40.4471
28	141.8	153.5	4.456	144.5	162.4	-11.6671
29	115.1	153.5	4.456	144.5	162.4	-38.3371
30	125.5	153.5	4.456	144.5	162.4	-27.9971
31	155.3	153.5	4.456	144.5	162.4	1.8229
32	117.3	153.5	4.456	144.5	162.4	-36.1871
33	133.2	153.5	4.456	144.5	162.4	-20.2171
34	144.3	153.2	4.412	144.3	162.0	-8.8462
35	134.1	153.2	4.412	144.3	162.0	-19.0262
36	134.2	153.2	4.412	144.3	162.0	-19.0062
37	134.9	153.2	4.412	144.3	162.0	-18.2762
38	142.6	153.2	4.412	144.3	162.0	-10.6162

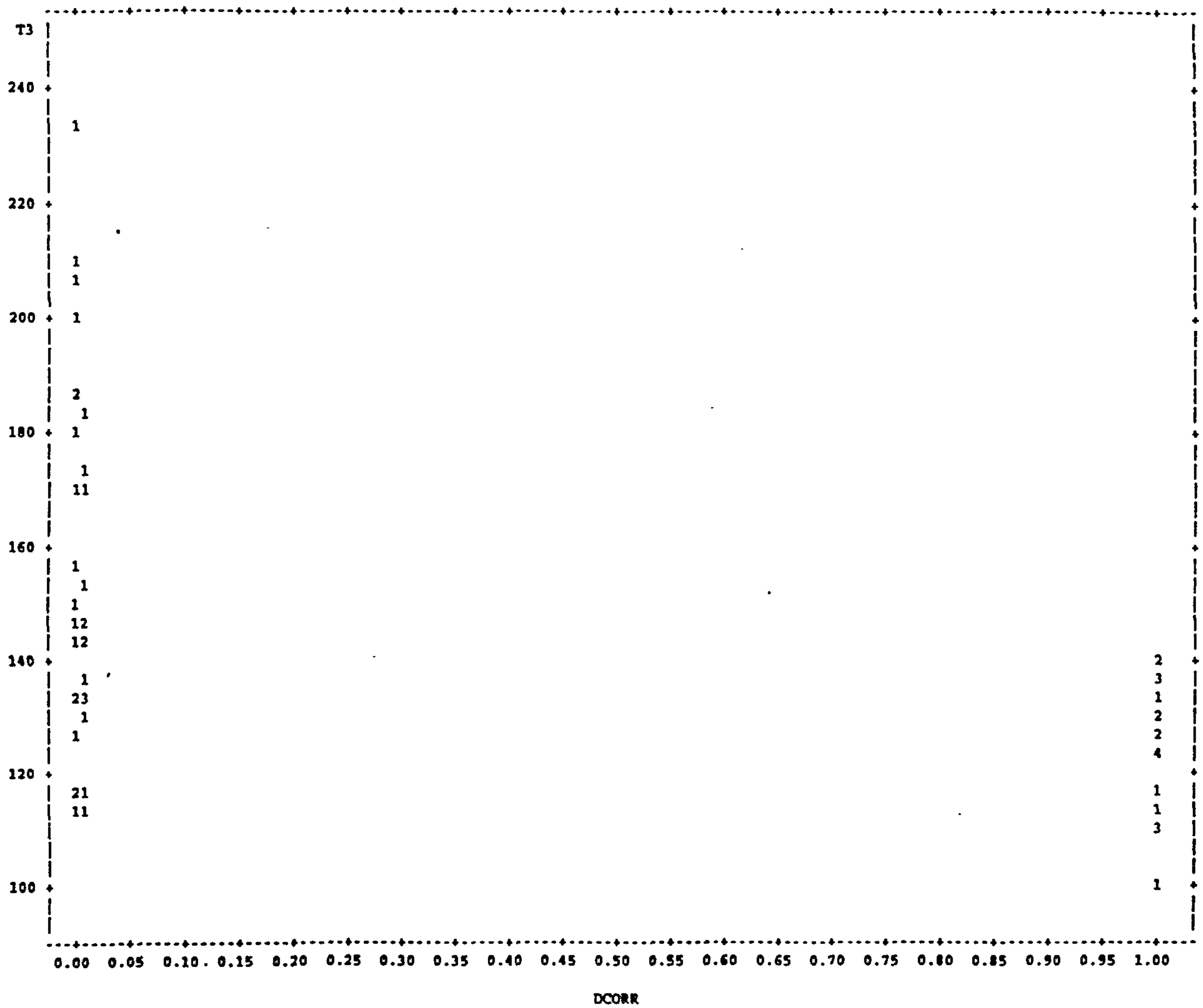
18	20.8700	15.6976	0.663	14.3659	17.0292	5.1724
19	18.7200	15.6976	0.663	14.3659	17.0292	3.0224
20	21.7500	15.6976	0.663	14.3659	17.0292	6.0524
21	16.1100	15.6976	0.663	14.3659	17.0292	0.4124
22	17.4800	15.6976	0.663	14.3659	17.0292	1.7824
23	18.4500	15.6976	0.663	14.3659	17.0292	2.7524
24	9.8900	12.7408	0.519	11.6993	13.7822	-2.8508
25	9.4900	12.7408	0.519	11.6993	13.7822	-3.2508
26	9.0000	12.7408	0.519	11.6993	13.7822	-3.7408
27	10.5100	12.7408	0.519	11.6993	13.7822	-2.2308
28	10.8600	12.7408	0.519	11.6993	13.7822	-1.8808
29	11.3000	12.7408	0.519	11.6993	13.7822	-1.4408
30	10.2700	12.7408	0.519	11.6993	13.7822	-2.4708
31	11.1500	12.7408	0.519	11.6993	13.7822	-1.5908
32	10.5700	12.7408	0.519	11.6993	13.7822	-2.1708

-- TMPT=15-18 --

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
33	11.3600	12.7408	0.519	11.6993	13.7822	-1.3808
34	11.8600	12.7703	0.514	11.7393	13.8014	-0.9103
35	11.8300	12.7703	0.514	11.7393	13.8014	-0.9403
36	10.3300	12.7703	0.514	11.7393	13.8014	-2.4403
37	10.0300	12.7703	0.514	11.7393	13.8014	-2.7403
38	13.4700	12.7703	0.514	11.7393	13.8014	0.6997
39	10.7800	12.7703	0.514	11.7393	13.8014	-1.9903
40	13.7100	12.7703	0.514	11.7393	13.8014	0.9397
41	11.4600	12.7703	0.514	11.7393	13.8014	-1.3103
42	11.3600	12.7703	0.514	11.7393	13.8014	-1.4103
43	12.3500	12.7703	0.514	11.7393	13.8014	-0.4203
44	13.0700	15.6976	0.663	14.3659	17.0292	-2.6276
45	12.3300	15.6976	0.663	14.3659	17.0292	-3.3676
46	10.1300	15.6976	0.663	14.3659	17.0292	-5.5676
47	12.0200	15.6976	0.663	14.3659	17.0292	-3.6776
48	13.4800	15.6976	0.663	14.3659	17.0292	-2.2176
49	11.7000	15.6976	0.663	14.3659	17.0292	-3.9976
50	12.4800	15.6976	0.663	14.3659	17.0292	-3.2176
51	13.1400	15.6976	0.663	14.3659	17.0292	-2.5576
52	14.9100	15.6976	0.663	14.3659	17.0292	-0.7876
53	12.6300	15.6976	0.663	14.3659	17.0292	-3.0676

Sum of Residuals	0
Sum of Squared Residuals	448.7963
Predicted Resid SS (Press)	487.8609

THPT=15-18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200
180
160
140
120
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DCORR

--- TMPT=92-95 ---

Model: MODEL1
Dependent Variable: T4

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	13.35356	13.35356	35.471	0.0001
Error	57	21.45854	0.37647		
C Total	58	34.81209			

Root MSE	0.61357	R-square	0.3836
Dep Mean	4.01864	Adj R-sq	0.3728
C.V.	15.26803		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	4.351631	0.09750241	44.631	0.0001
DCORR	1	-1.023240	0.17180734	-5.956	0.0001

Dependent Variable: T3

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	21318.12087	21318.12087	60.328	0.0001
Error	57	20142.20249	353.37197		
C Total	58	41460.32336			

Root MSE	18.79819	R-square	0.5142
Dep Mean	149.58847	Adj R-sq	0.5057
C.V.	12.56660		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|

11	3.2200	4.3414	0.097	4.1481	4.5347	-1.1214
12	4.2200	4.3414	0.097	4.1481	4.5347	-0.1214
13	4.7000	4.3414	0.097	4.1481	4.5347	0.3586
14	4.2300	4.3414	0.097	4.1481	4.5347	-0.1114
15	4.1200	4.3414	0.097	4.1481	4.5347	-0.2214
16	4.7600	4.3414	0.097	4.1481	4.5347	0.4186
17	4.3800	4.3414	0.097	4.1481	4.5347	0.0386
18	4.4900	4.3414	0.097	4.1481	4.5347	0.1486
19	4.6500	4.3414	0.097	4.1481	4.5347	0.3086
20	4.7900	4.3414	0.097	4.1481	4.5347	0.4486
21	3.8700	3.3284	0.141	3.0465	3.6103	0.5416
22	3.1400	3.3284	0.141	3.0465	3.6103	-0.1884
23	3.8400	3.3284	0.141	3.0465	3.6103	0.5116
24	3.3200	3.3284	0.141	3.0465	3.6103	-0.00839
25	3.5900	3.3284	0.141	3.0465	3.6103	0.2616
26	2.7900	3.3284	0.141	3.0465	3.6103	-0.5384
27	3.2000	3.3284	0.141	3.0465	3.6103	-0.1284
28	3.6100	3.3284	0.141	3.0465	3.6103	0.2816
29	3.8200	3.3284	0.141	3.0465	3.6103	0.4916
30	3.4900	3.3284	0.141	3.0465	3.6103	0.1616
31	3.7300	4.3516	0.098	4.1564	4.5469	-0.6216
32	4.3900	4.3516	0.098	4.1564	4.5469	0.0384
33	4.1100	4.3516	0.098	4.1564	4.5469	-0.2416
34	4.3800	4.3516	0.098	4.1564	4.5469	0.0284
35	4.7000	4.3516	0.098	4.1564	4.5469	0.3484
36	4.1000	4.3516	0.098	4.1564	4.5469	-0.2516
37	5.2800	4.3516	0.098	4.1564	4.5469	0.9284
38	4.7500	4.3516	0.098	4.1564	4.5469	0.3984
39	4.2500	4.3516	0.098	4.1564	4.5469	-0.1016
40	4.7200	4.3516	0.098	4.1564	4.5469	0.3684
41	3.6100	4.3414	0.097	4.1481	4.5347	-0.7314
42	3.7300	4.3414	0.097	4.1481	4.5347	-0.6114
43	3.6000	4.3414	0.097	4.1481	4.5347	-0.7414
44	3.3600	4.3414	0.097	4.1481	4.5347	-0.9814
45	3.1800	4.3414	0.097	4.1481	4.5347	-1.1614
46	3.3000	4.3414	0.097	4.1481	4.5347	-1.0414
47	3.1000	4.3414	0.097	4.1481	4.5347	-1.2414
48	3.4600	4.3414	0.097	4.1481	4.5347	-0.8814
49	4.2900	4.3414	0.097	4.1481	4.5347	-0.0514
50	3.5800	4.3414	0.097	4.1481	4.5347	-0.7614
51	3.0200	3.3284	0.141	3.0465	3.6103	-0.3084
52	3.1000	3.3284	0.141	3.0465	3.6103	-0.2284

1 Subchronic Rat Perchlorate Study, Correlations with external dose 57

10:02 Monday, February 8, 1999

-- TMPT=92-95 --

Obs	Dep Var T4	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual

33	156.3	162.9	2.987	156.9	168.9	-6.5931
34	184.1	162.9	2.987	156.9	168.9	21.1569
35	162.7	162.9	2.987	156.9	168.9	-0.1931
36	174.6	162.9	2.987	156.9	168.9	11.6669

---------- TMPT=92-95 ----------

Obs	Dep Var T3	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
37	178.4	162.9	2.987	156.9	168.9	15.4869
38	157.3	162.9	2.987	156.9	168.9	-5.5531
39	154.0	162.9	2.987	156.9	168.9	-8.8631
40	148.7	162.9	2.987	156.9	168.9	-14.1731
41	161.1	162.5	2.957	156.6	168.4	-1.3843
42	133.7	162.5	2.957	156.6	168.4	-28.7843
43	153.4	162.5	2.957	156.6	168.4	-9.0643
44	140.3	162.5	2.957	156.6	168.4	-22.1943
45	122.9	162.5	2.957	156.6	168.4	-39.5843
46	126.2	162.5	2.957	156.6	168.4	-36.2543
47	136.1	162.5	2.957	156.6	168.4	-26.3843
48	158.9	162.5	2.957	156.6	168.4	-3.6343
49	134.9	162.5	2.957	156.6	168.4	-27.5443
50	163.8	162.5	2.957	156.6	168.4	1.2957
51	122.9	122.0	4.312	113.4	130.6	0.8509
52	116.1	122.0	4.312	113.4	130.6	-5.9191
53	116.6	122.0	4.312	113.4	130.6	-5.4191
54	107.7	122.0	4.312	113.4	130.6	-14.2791
55	135.3	122.0	4.312	113.4	130.6	13.2909
56	117.3	122.0	4.312	113.4	130.6	-4.7191
57	137.2	122.0	4.312	113.4	130.6	15.1809
58	124.0	122.0	4.312	113.4	130.6	2.0209
59	125.6	122.0	4.312	113.4	130.6	3.6009

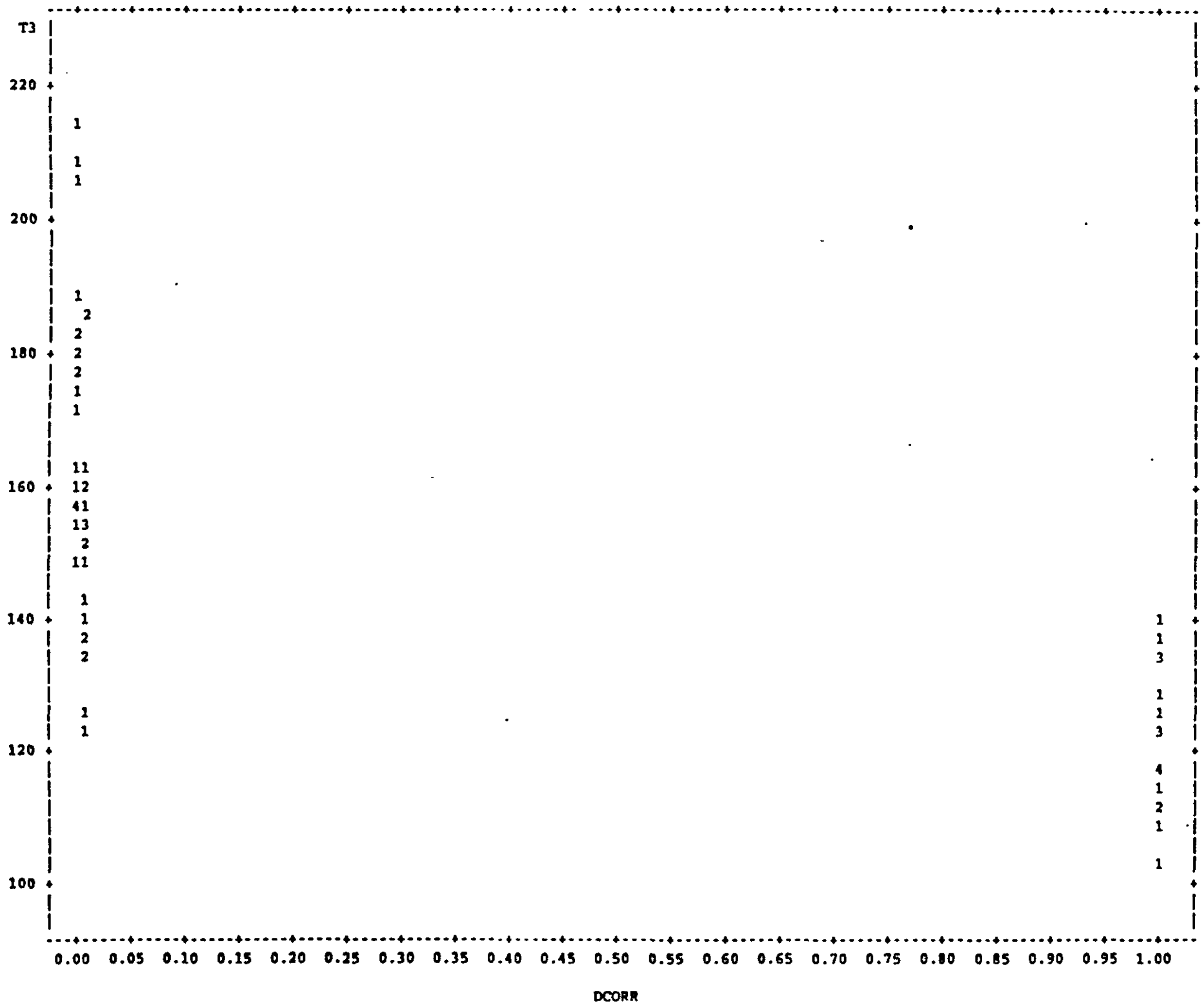
Sum of Residuals	0
Sum of Squared Residuals	20142.2025
Predicted Resid SS (Press)	21311.0806

Obs	Dep Var TSH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Residual
1	15.7100	16.5623	0.255	16.0518	17.0727	-0.8523
2	14.3800	16.5623	0.255	16.0518	17.0727	-2.1823
3	15.1800	16.5623	0.255	16.0518	17.0727	-1.3823
4	17.7200	16.5623	0.255	16.0518	17.0727	1.1577
5	17.5000	16.5623	0.255	16.0518	17.0727	0.9377

47	15.8900	16.5804	0.252	16.0751	17.0857	-0.6904
48	16.6400	16.5804	0.252	16.0751	17.0857	0.0596
49	14.4500	16.5804	0.252	16.0751	17.0857	-2.1304
50	15.7300	16.5804	0.252	16.0751	17.0857	-0.8504
51	17.7500	18.3730	0.368	17.6361	19.1099	-0.6230
52	18.8100	18.3730	0.368	17.6361	19.1099	0.4370
53	16.2600	18.3730	0.368	17.6361	19.1099	-2.1130
54	19.2000	18.3730	0.368	17.6361	19.1099	0.8270
55	18.7600	18.3730	0.368	17.6361	19.1099	0.3870
56	15.6900	18.3730	0.368	17.6361	19.1099	-2.6830
57	18.2700	18.3730	0.368	17.6361	19.1099	-0.1030
58	14.9700	18.3730	0.368	17.6361	19.1099	-3.4030
59	18.9300	18.3730	0.368	17.6361	19.1099	0.5570

Sum of Residuals 0
Sum of Squared Residuals 146.6680
Predicted Resid SS (Press) 157.1575

TMPT=92-95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DCORR

Has Perchlorate in Drinking Water Increased the Rate of Congenital Hypothyroidism?

Running Title: Perchlorate and Congenital Hypothyroidism

Word Count: 777

Steven H. Lamm, MD & Martha Doemland, PhD

From Consultants in Epidemiology and Occupational Health, Inc. (CEOH, Inc.), Washington, DC (Drs. Lamm and Doemland)

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Journal of Occupational and Environmental Medicine
In press (January 22, 1999)

Abstract

Perchlorate, known to inhibit the human thyroid at doses above 200 mg/day, was detected in the drinking water supplies of seven counties in California and Nevada at levels of 4 to 16 ug/l in 1997. The data from the neonatal screening programs of the state health departments were analyzed for any increased incidence of congenital hypothyroidism in those counties. County-specific, ethnicity-specific data for Nevada and California were obtained for 1996-1997. Within these seven counties, nearly 700,000 newborns had been screened. 249 cases were identified, where 243 were expected, for an over all risk ratio of 1.0 (95% confidence interval, 0.9-1.2). The risk ratios for the individual counties ranged between 0.6 and 1.1. These data in this ecological analysis do not indicate any increase in the incidence of congenital hypothyroidism with the reported perchlorate levels.

Keywords: Perchlorate, thyroid, congenital hypothyroidism

Introduction

Congenital hypothyroidism is a preventable cause of mental retardation and is detected at birth through neonatal screening programs. Perchlorate, now a known environmental contaminant or drinking and surface waters, is known to block thyroid hormone formation by competitively inhibiting the uptake of iodine by the thyroid gland. An analysis has been conducted to determine whether the counties with perchlorate-containing water have an increased rate of congenital hypothyroidism.

Methods

The source of perchlorate contamination in California and Nevada originated from industrial sites manufacturing or using perchlorate for missiles, rockets, or fireworks. An industrial site in Nevada led to contamination of Lake Mead which contaminated the Colorado River water supply for Southern California and the water supply for Las Vegas (Clark County) Nevada at levels of 5-8 ppb and up to 16 ppb, respectively. The US Environmental Protection Agency (EPA) Region 9 has identified six counties in California and one in Nevada with perchlorate in the drinking water supply. (Figure I)

The health departments of both Nevada and California have conducted neonatal screening programs for congenital hypothyroidism for over ten years. A heel-stick blood sample of all newborns is used to assess the presence of a variety of congenital metabolic diseases. Participation is mandatory and covers all hospitals with birthing units. Follow-up after diagnosis and referral for treatment is supervised by the state health departments.

The county-specific congenital hypothyroidism case counts and live birth counts for 1996 and 1997 have been obtained for both California and Nevada. Theses data were supplied by the respective state Health Departments (George Cunningham, MD, MPH, Chief of the Genetics Disease Branch, Primary Care & Family Health Division, California Department of Health Services; Gloria Dayhli, Bureau of Family Health Services, Nevada State Health Division). The California data were stratified by ethnicity since fifty percent of California births are Hispanic and Hispanic ethnicity has been shown to be a risk factor for congenital hypothyroidism.

Results

California and Nevada comprise a population of about 35 million people with a birth rate of about 16%. The neonatal screening programs cover essentially one hundred percent of the live births in each state, including the 700,000 newborns who were screened during 1996-97 in the seven counties with perchlorate-contaminated drinking water.

Based on state incidence rates of congenital hypothyroidism, 243 cases would have been expected in the seven county area during 1996-1997 and 249 cases were observed [Table 1]. This risk ratio is 1.02 (95% confidence limits, 0.9-1.2). The risk ratios (congenital hypothyroidism standardized birth prevalence ratio) for each county was calculated for the individual counties ranged between 0.6 and 1.1. Thus, in Nevada and California, the counties with detectable levels of perchlorate in the drinking water have prevalence rates for congenital hypothyroidism than do not differ from the expected based on state rates.

Nearly the entire water supply for Clark County, Nevada comes from the primary source of perchlorate contamination (Lake Mead). The California counties have more spotty and intermittent exposure. Nonetheless, this ecological examination of the congenital hypothyroidism data (1996-97) shows no increase in the prevalence of congenital hypothyroidism in counties with detected perchlorate levels in the drinking water.

Discussion

Perchlorate was detected in the range of 4-16 ppb (μg/l) in drinking water supplies for California and Nevada. Assuming water intake of two liters per day, this might provide a daily dosage of perchlorate of approximately 20 μg per day. A daily intake rate of perchlorate at 20 μg per day can be compared with the minimum effective dose of 200 mg/day (200,000 μg/day) that has been used medically to suppress the thyroid in treatment of hyperthyroidosis.

Congenital hypothyroidism occurs when both the maternal thyroid and the fetal thyroid are unable to supply adequate thyroid hormone to the fetus. This occurs endemically only in the presence of severe iodine deficiency, a condition rarely known in the United States, and sporadically with structural or metabolic defects in the thyroid. Children born without a thyroid have normal intellect if thyroid treatment starts early [3] because the maternal thyroxine that crosses the placenta is usually sufficient to sustain the fetus. [4, 5] Even moderate iodine deficiency in a population yielded only transient changes in thyroid hormone levels (T_4, TSH) and no increase in congenital hypothyroidism.[6]

Comparison of the county-specific rates of congenital hypothyroidism (based on prevalence rates derived from mandatory reporting programs) in California and Nevada reveal that counties with detected levels of perchlorate in the drinking water do not have higher rates of congenital hypothyroidism. These data, at an ecological level of analysis, seem to indicate that no increased rate of congenital hypothyroidism is associated with the levels of perchlorate found in the drinking waters of California and Nevada.

References

[1] Environmental Protection Agency. Office of Ground Water and Drinking Water. Perchlorate. December 15, 1998.

[2]Lorey FW and Cunningham GC. Birth Prevalence of Primary Congenital Hypothyroidism by Sex and Ethnicity. Human Biology, Aug 1992; 64(4): 531-538.

[3]Burrow GN, Fisher DA, and Larsen PR. Mechanisms of Disease: Maternal and Fetal Thyroid Function. NEJM, 1994 Oct 20; 331(16): 1072-1078.

[4]Vulsma T, Gons MH, de Vijlder JJ. Maternal-Fetal Transfer of Thyroxine in Congenital Hypothyroidism due to a Total Organification Defect or Thyroid Agenesis. NEJM, 1989 Jul 6; 321(1):13-16.

[5]Larsen PR. Maternal Thyroxine and Congenital Hypothyroidism. NEJM, 1989 Jul 6; 321(1): 44-46.

[6]Delange F, Heidemann P, Bourdoux P, Larsson A, Vigneri R, Klett M, Beckers C, and Stubbe P.: Regional variations of iodine nutrition and thyroid function during the neonatal period in Europe. Biol. Neonate, 1986; 49: 322-330.

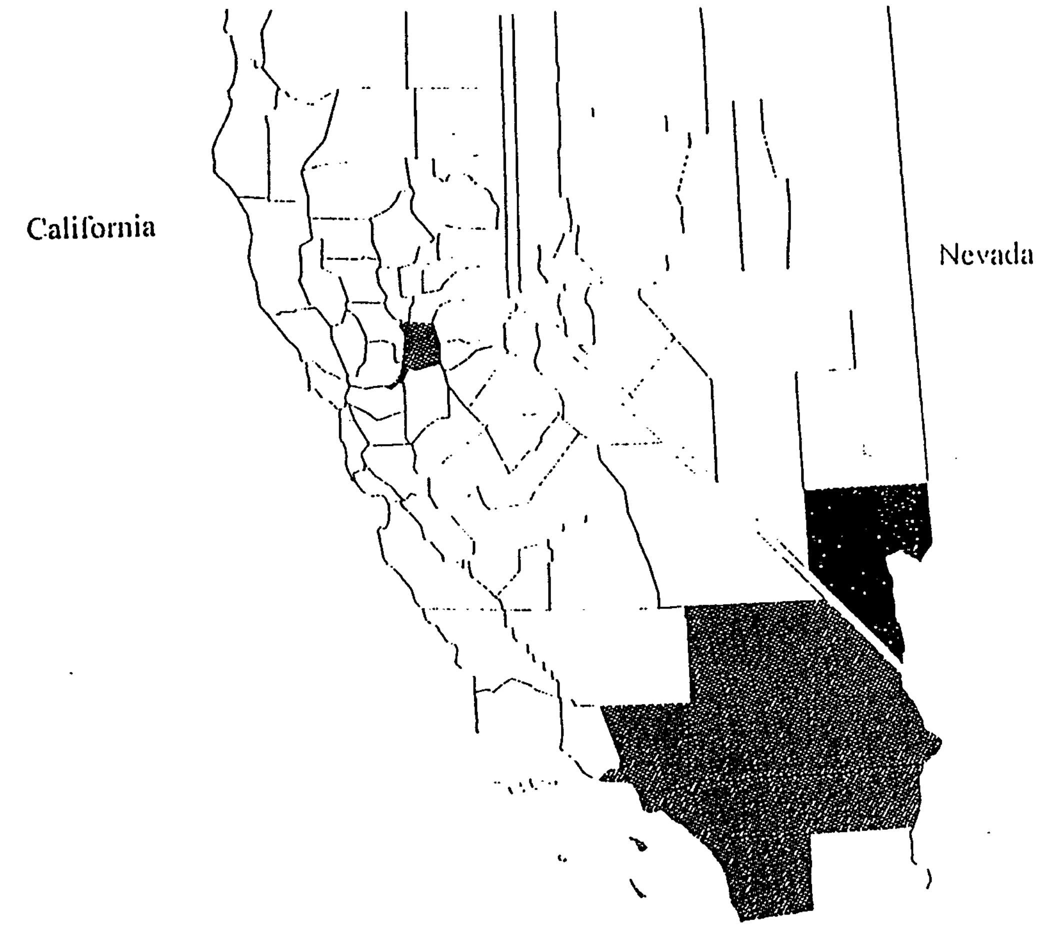
Table 1

Congenital Hypothyroidism Cases (Observed and Expected*) for 1996 and 1997 in Nevada and California Counties with Perchlorate Reported in Water Supply

State	County	Newborns Number Screened	Congenital Hypothyroidism Cases Observed	Expected	Observed/Expected	95% Conf. Limits
Nevada	Clark	36,016	7	8.3	0.84	(0.34-1.74)
California	Los Angeles	338,934	136	123.5	1.10	(0.92-1.30)
	Orange	101,227	40	35.9	1.12	(0.80-1.52)
	Riverside	43,577	11	15.6	0.71	(0.35-1.26)
	Sacramento	39,235	8	12.9	0.62	(0.27-1.22)
	San Bernardino	51,637	17	18.4	0.92	(0.54-1.48)
	San Diego	80,582	30	28.2	1.06	(0.72-1.52)
	Total	655,192	242	234.6	1.03	(0.90-1.16)
All seven counties		691,208	249	42.9	1.03	(0.90-1.16)

* Expected numbers have been adjusted for Hispanic ethnicity.

Figure 1. Counties in California and Nevada with Perchlorate Detected in Drinking Water



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January 21, 1999

Annie M. Jarabek
National Center for Environmental Assessment (NCEA)
Environmental Protection Agency (MD-52)
3210 Highway 54 Room 320
Research Triangle Park, NC 27709

Dear Annie,

On September 1, 1998, you received from us a peer-reviewed report of our perchlorate worker occupational health study entitled "Thyroid Health Status of Ammonium Perchlorate Workers: A Cross-Sectional Occupational health Study". You had asked that we notify when it had been submitted for publication. We did, and we sent you a copy of the submitted version. You asked that we inform you if and when the paper is accepted by the journal.

This is to notify you that the editor of the Journal of Occupational and Environmental Medicine (JOEM), Paul W. Brandt-Rauf, MD, ScD, DrPH, has informed me that the paper has been accepted for publication in the JOEM. Attached is the as-accepted version of that paper. We hope that it will be found useful to your process to assess the risks to human health from exposure to perchlorate. Please let us know if there are any questions that either you or your associates have for us.

Cordially,

Steven H. Lamm, MD DTPH

Sent by Federal Express

Cc: Linda Ferguson - AMPAC

Thyroid Health Status of Ammonium Perchlorate Workers:

A Cross-Sectional Occupational Health Study

Running title: Thyroid Function in Perchlorate Workers (39 spaces)

By

Steven H. Lamm, MD, Lewis E. Braverman, MD, Feng Xiao Li, MD, DrPH,
Kent Richman, PhD, Sam Pino, BSc, and Gregory Howearth, BSc

From Consultants in Epidemiology and Occupational Health, Inc. (CEOH, Inc.), Washington, DC (Dr. Lamm, Dr. Li), Department of Medicine, Brigham and Women's Hospital, Boston, MA (Dr. Braverman, Mr. Pino), and American Pacific Corporation, Cedar City, UT (Dr. Richman, Mr. Howearth).

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4. Kent Richman, PhD, - Director Analytical Labs and Product Development, American-Pacific Corporation
5. Sam Pino, BSc, - Director of Iodine Research Laboratory, Brigham & Women's Hospital
6. Gregory Howearth, BSc - Safety and Environmental Officer, American-Pacific Corporation

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Abstract

Since pharmaceutical exposures to perchlorate are known to suppress thyroid function in patients with hyperthyroidism, a study of employees at a perchlorate manufacturing plant has been conducted to assess whether occupational exposure to perchlorate suppresses thyroid function. Exposure to perchlorate was assessed by measurement of ambient air concentrations of total and respirable perchlorate particles, and systemic absorption was assessed by measurement of urinary perchlorate excretion. Airborne exposures ranged from 0.004 to 167 mg/day total particulate perchlorate. Urinary perchlorate measurements demonstrated that exposure to the airborne particulate perchlorate resulted in systemic absorption. Workers were in four exposure groups with mean perchlorate absorbed dosages of 1, 4, 11 and 34 mg perchlorate per day. Thyroid function was assessed both by TSH, FTI, T_4, T_3, THBR, or TPO antibodies and by clinical examination. No differences in thyroid function parameters were found between the four groups of workers across about three orders of magnitude of exposure and of dose. Thus, the human thyroid function was not affected by these levels of absorbed perchlorate. In addition, no clinical evidence of thyroid abnormalities was found in any exposure group. The blood cell counts were normal in all groups indicating no evidence of hematotoxicity in this exposure range. The absence of evidence of an effect on thyroid function or blood cells from occupational airborne perchlorate exposure at a mean absorption of 34 mg/day demonstrates a human no observed adverse effect level that can assist in the evaluation of human health risks from environmental perchlorate contamination.

Introduction

This occupational health study was conducted in July of 1998 at the only US industrial site currently manufacturing ammonium perchlorate (the Cedar City, Utah site of American Pacific Corporation). The purpose was to assess the health status, specifically thyroid function, of workers with long-term (months to years) exposure to perchlorate. This study included measures of exposure to perchlorate particulates, measures of urinary perchlorate to determine the magnitude of systemic absorption from perchlorate particulates, and measures of thyroid function to determine whether (and if so, to what degree) the thyroid function was affected by the perchlorate exposure. While this study was primarily designed to develop a health assessment of occupationally exposed perchlorate workers, the information gathered may also be useful in assessing health risks to persons environmentally exposed to perchlorate.

Perchlorates have been used industrially for over 50 years in propellants and explosives as oxidizers because of the strong oxidizing potential of their salts. Ammonium perchlorate is used as the oxidizer in solid propellant for rockets and missiles, such as the boosters for the space shuttle and the Titan rocket, as well as for fireworks. Other perchlorate salts include sodium perchlorate, which is used as an oxidizer in slurry explosives manufacturing, and potassium perchlorate, which is used in road flares and in air bag inflation systems. Perchlorate salts are highly soluble in water. Perchlorate ions have been detected in ground and surface waters near sites where perchlorates are used or manufactured. The perchlorate salts fully ionize, and the perchlorate ion (ClO_4^-) persists for several decades in surface and ground waters. Recent improvements in the laboratory method for detecting perchlorate in drinking-water supplies have lowered the limit of detection from 400 parts per billion (ppb) to 4 ppb. Subsequent surveys of water supplies in California have detected perchlorate in a number of wells, the sources of which have been traced back to various sites of industrial perchlorate use, and in a major water supply (the Colorado river, downstream from Lake Mead), the source of which has been traced back to an area of perchlorate manufacture in Nevada. Some of the well measurements in California have exceeded 18 ppb, and the water measurements from the Colorado River have ranged from 5-8 ppb. The potential health risks from short-term and long-term consumption of such levels of perchlorate in drinking water supplies are currently under assessment by the US Environmental Protection Agency.

Although thyroid function is dependent upon an adequate dietary intake of iodine (100-200 μg/day) as substrate for hormone synthesis, the thyroid readily compensates for a modest decrease in iodine intake by enlarging and actively transporting a larger fraction of the circulating iodine. An Austrian study showed that euthyroid subjects (n=2,308) had normal levels of the thyroid hormone thyroxine (T_4) in spite of having mildly low iodine intakes (as indicated by urinary excretion of < 100 μg I per gram creatinine).[1]

Studies in humans and rodents demonstrate that the primary effect of perchlorate is to block the uptake of iodine by the thyroid gland, thus potentially decreasing the production of the thyroid hormones T_4 and triiodothryronine (T_3). Perchlorate (as potassium perchlorate) has been used medically since the late 1950s to treat hyperthyroidism, and its effects on the thyroid have been well studied.[2] It is a competitive inhibitor of the sodium/iodide symporter of the thyroid follicular cell, which actively transports iodine from the blood into the thyroid. It does not appear to have an important effect on thyroid hormone synthesis. Perchlorate is the most effective drug for blocking the thyroidal uptake of iodine. It is excreted unmetabolized, with approximately 95% recovery in urine over 72 hours.[3] Eichler reported that within 6-8 hours the urine contained 50% of a 1 or 2 gram oral dose given to an adult male.[3] Similarly, Durand reported that within 5-9 hours the urine contained 50% of a 0.8 gram oral dose given to an adult male.[4]

In 1952, Stanbury and Wyngaarden demonstrated the effectiveness of perchlorate in treating hyperthyroidism due to Graves' Disease at dose levels of 200 mg potassium perchlorate three times daily.[5] Subsequently, treatment doses were increased to 1,200 mg per day to accelerate the induction of normal thyroid function. Following case reports of fatal aplastic anemia or agranulocytosis in patients treated with perchlorate at doses of 600-1,600 mg/day for extended periods, the use of perchlorate for treating Graves' disease markedly decreased. More recently, Wenzel and Lente treated hyperthyroid patients with Graves' disease with perchlorate at doses of 900 mg or below daily for 1 year with excellent results and no serious side effects.[6] Perchlorate is now used to treat patients who have iodine-induced thyrotoxicosis (e.g., amiodarone-associated thyrotoxicosis [AAT]). Amiodarone, a potent drug used to treat cardiac tachyarrythmias, contains nearly 40 percent iodine. AAT patients treated for one month or longer with perchlorate at doses up to 1,000 mg/day perchlorate had no evidence of agranulocytosis or aplastic anemia.[7,8,9,10]

Few studies have been conducted on the effect of perchlorate on healthy human subjects. In one study, Burgi et al. showed in five healthy volunteers that 600 mg/day was sufficient to completely block iodide uptake by the thyroid.[11] In another study, Brabant et al. were unable in five healthy male volunteers to induce a state of iodine depletion by administering orally 900 mg/day of potassium perchlorate for four weeks.[12] Brabant is reported to have observed mild goiters without an increase in TSH levels in a five week long repeat of that study. These are the only studies that indicate toxicity levels of perchlorate exposure in healthy humans.

The present occupational health study focuses on thyroid health status and was conducted in an ammonium perchlorate manufacturing plant. The manufacturing process at this plant begins with the electrolysis of brine (sodium chloride in water) to first form sodium chlorate ($NaClO_3$) and then sodium perchlorate ($NaClO_4$). Ammonium chloride (NH_4Cl) is formed from ammonia (NH_3) and hydrochloric acid (HCl). The sodium perchlorate is reacted with the ammonium chloride to form ammonium perchlorate (NH_4ClO_4) and salt (NaCl). The solution is cooled, and the ammonium perchlorate crystals are dried and blended to specifications.

Study Design

This was a cross-sectional study of two similar worker populations from the same industrial complex - ammonium perchlorate production workers and sodium azide production workers; the latter served as a control group. The purpose was to assess perchlorate exposure and thyroid function in both groups. The two production plants are in close proximity, and the workers share locker facilities, storage areas, training and administrative areas, etc., but not production areas.

Perchlorate exposure was measured using full-shift breathing zone air sampling for both total perchlorate particles and respirable perchlorate particles. Urinary perchlorate concentration was assessed at both the beginning and end of the twelve-hour shift in which the particulate exposure was measured. Particle-size-selective sampling was conducted to obtain the mass mean aerodynamic diameter of the particles.

Thyroid function was assessed by measuring serum thyroid stimulating hormone (TSH), T_4, and T_3 concentrations, the thyroid hormone binding ratio (THBR), and the free T_4 index (FTI). Thyroid peroxidase (TPO) antibody concentrations were measured to identify workers with underlying Hashimoto's thyroiditis. If the occupational exposure to perchlorate were suppressing the thyroid by blocking iodine uptake, the expected observation would be that the TSH levels would increase.

Urinary iodine concentrations were obtained to determine if workers had adequate iodine intake. Blood samples for complete blood counts (CBC) and serum samples for a chemistry panel were also obtained. Physical exams and medical histories with careful thyroid evaluation were performed on all subjects.

Materials and Methods

A. Study Population

The perchlorate production plant is located in the industrial facility of the American Pacific Corporation in Iron County, Utah, west of Cedar City. The facility, which began production in 1989, employs approximately 190 workers in four major divisions. The thirty-nine employees assigned to direct perchlorate production and the twenty-one employees assigned to direct azide production were eligible to be study participants. Employees assigned to administrative, engineering, maintenance, and supervisory positions were not eligible to be study participants. Fifty-eight of the sixty employees eligible for participation did participate. Two of the eligible perchlorate workers were not at the plant at the time of the study because of vacation or military duty and did not participate. The production employees work 12-hour shifts (on three days; off three days), with rotation from days to night approximately monthly. The employee population from both plants are similar in that they are drawn from the same population base, have the same management procedures and policies, work similar rotating shifts, and have participated in prior medical monitoring programs at the facility.

All participants were instructed as to the nature of the study and informed consent was obtained. The study protocol and consent forms were approved by the Georgetown University School of Medicine institutional review board. Pre-shift and post-shift urine samples were obtained from all participants, and post-shift blood samples were obtained from all but one participant who declined to give a blood sample. Air sampling equipment was used throughout the shift for determining both total and respirable perchlorate particle exposure. Participants completed a medical questionnaire and underwent a physical examination conducted by a local physician's assistant and a thyroid examination conducted by a thyroid specialist (LEB). Examiners were not aware of a participant's study group. Laboratory samples were prepared with participant code numbers to keep the laboratory personnel blinded as to a participant's study group. The personnel and director of each laboratory maintained quality control and assurance procedures.

B. Perchlorate exposure groups

The job assignments of the perchlorate production workers were classified into three categories of presumptive exposure (low, medium, and high) based on the visible dust generated. The categories of low, medium and high were used as follows to classify workers:

A- Low: employees handling only solutions or slurries of perchlorates. This includes electrolysis through crystallization processes.

B- Medium: employees handling limited quantities of dry perchlorates, resulting in only minor visible-dust exposure. This includes the initial drying process.

C- High: employees handling large quantities of dry perchlorates, resulting in significant visible-dust exposure. This includes the blending and packaging operations.

C. Urine samples

Pre- and post-shift urine samples were collected from all participants to measure urinary perchlorate, iodine, and creatinine levels. Urine samples were frozen after collection and thawed prior to analysis. Urinary creatinine and iodine measurements were performed in the Iodine Research Laboratory at the Brigham & Women's Hospital (Boston, MA), using the Jaffee alkaline picrate method for creatinine and the Sandell-Koltoff reaction for iodine.[13] Urinary perchlorate measurements were performed by Dr. Kent Richman at the American Pacific Corporation laboratory, using a US Air Force developed modification of a Dionex conductivity detection method. The method (available on request) is capable of measuring urinary perchlorate concentrations of 0.5 parts per million (ppm) or greater.[14]

D. Blood samples

Post-shift blood samples were collected. Complete blood counts were performed at the clinical laboratories of Valley View Medical Center, Cedar City, Utah. The complete blood count included absolute counts of red blood cells, white blood cells, and platelets; absolute and relative counts of lymphocytes, neutrophiles, monocytes, eosinophiles, and basophiles; and hemoglobin, hematocrit, and red cell parameters (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration).

E. Serum samples for thyroid function studies

Thyroid function studies on post-shift serum samples were performed in the Endocrine-Hypertension Research Laboratory of Brigham & Women's Hospital. Thyroid function studies were carried out in duplicate, in the same assay, and in random order. Tests and their methods follow with the normal values for the laboratory indicated in parentheses. TSH [thyroid stimulating hormone] (0.45-4.5 μU/ml) was measured by chemiluminescence, (Beckman Access, Chaska, MN), T_4 [thyroxine] (5-11 μg/dl) and THBR [thyroid hormone binding ratio] (0.85-1.10) by radioimmunoassay (Diagnostic Products Corp, Los Angeles, CA), T_3 [triiodotyronine] (87-178 ng/dl) by radioimmunoassay (Beckman Access, Chaska, MN), and TPO [thyroid peroxidase] (<20 IU/ml) by ELISA (American Laboratory Products Co, LTD). The FTI [free thyroxine index] is the product of the T_4 concentration and the THBR. The results reported for each subject are the mean of the duplicate values for each test.

F. Serum sample for blood chemistry panel.

Post-shift serum samples obtained from the participants were collected for analysis of a chemistry panel by Quest Diagnostics of Cambridge, MA . The chemistry panel included serum levels of calcium, phosphate, glucose, blood urea nitrogen, creatinine, uric acid, cholesterol, total protein, albumin, alkaline phosphatase, lactic dehydrogenase, SGOT, and total bilirubin.

G. Air sampling

Full-shift air sampling for total (< 40 μm) and for respirable (< 10 μm) breathing zone particles was carried out under the direction of David Houck, CIH. For total particulate, 5 μm PVC filters in 37-mm three-piece, closed-face cassettes were used, with a sampling rate of about two liters per minute and a sampling duration of ten to eleven hours. For respirable particulates, SKC aluminum cyclones were used with 5 μm PVC filters in 37-mm cassettes, at a flow rate of 1.9 liters per minute.

Montgomery-Watson Laboratories analyzed the cassettes for perchlorate. Cassette samples were dissolved in a 10-ml aliquot of 30 mM sodium hydroxide, and perchlorate concentration was measured using the California Department of Health Services analytic method for perchlorate in drinking water samples.

Size-selective sampling of airborne dust was performed during a production period with a Marple 8-stage Cascade Impactor in the blender building at a height of five feet above the floor in the area where the perchlorate C group worked. Particle size distributions were determined at the following eight cutpoints: 21.3 μm, 14.8 μm, 9.8 μm, 6.0 μm, 3.5 μm, 1.55 μm, 0.93 μm, and 0.52 μm. Dust particles in the range of 0.1 to 10 μm are generally considered to be "respirable", as they may enter and be retained by the deep regions of the lung. Total particle mass was also determined since the highly soluble perchlorate particle may be readily absorbed after deposition into the nasal passages or upper respiratory tract. Inhalable particles which may also precipitate in the upper areas were not separately measured. The mass mean aerodynamic diameter of the particles was calculated.

H. Urinary perchlorate excretion

To determine the time course of urinary perchlorate excretion, two workers were monitored for six days, with urine samples submitted every twelve hours. These employees worked in the high exposure area during the first twelve hours of each of the first three days; in the next three days they were assigned to the administrative building rather than the production area. Thus, the observation period includes three exposure periods followed by 3 ½ days of observation with no known exposure. The urinary perchlorate levels during the three unexposed days provide an indication of perchlorate elimination rates under the conditions of exposure experienced by these two workers.

I. Methods of Statistical Analysis

Statistical analysis of data was conducted using the Stata package for the personal computer. A t-test was applied to mean differences in all continuous exposure, outcome and demographic variables. Descriptive statistics for both exposure and outcome variables were calculated and presented as arithmetic and/or geometric means and standard deviations, medians, ranges (minimum and maximum), and interquartile ranges (25^{th} percentile and 75^{th} percentile). For categorical variables, a chi-square statistic was used. Two-tailed p values were calculated for each comparison. The absence of a statistically significant difference was inferred if the two-tailed p value was not less than 0.05. For outcome data, pair-wise t-tests were performed between the comparison group and each of the exposed groups. A non-parametric z-test for trend across ordered groups was conducted.[15]

Results

A. Population Description

A total of 58 employees participated in this occupational health study - 37 from the ammonium perchlorate production plant (35 male and 2 female) and 21 from the sodium azide production plant (19 male and 2 female), ranging in age from 20 to 56 years. The mean age of the ammonium perchlorate workers was 30 years compared to 35 years in the azide workers. Forty percent of the ammonium perchlorate production workers and fifty percent of azide production workers had been employed for more than five years.

B. Medical examination and questionnaire findings

No differences were found between the azide workers and the perchlorate workers or among the three perchlorate worker groups in the findings from their medical examinations or their responses on the medical questionnaire. Mean heights and weights were similar. The groups did not differ in their clinical findings (blood pressure, pulse, examination of body systems) from the medical examination. According to their answers on the medical questionnaire, the groups did not differ in alcohol or tobacco use, in medication use, in frequencies of family history of major systemic diseases (diabetes, hypertension, rheumatoid arthritis, thyroid disease, or cancer), or in reported medical problems.

Thyroid disease was identified in two workers. One worker in the low perchlorate exposure group (A) had been previously diagnosed with Grave's disease. His disease was diagnosed nine years prior to this employment and had been treated with radioactive iodine. He was now found to be hypothyroid and undermedicated. Previously undiagnosed thyroid disease was found in only one worker, a worker in the medium perchlorate exposure group (B) who was found in this examination to have an autoimmune condition of the thyroid, euthyroid Hashimoto's thyroiditis. Other than the first worker, no worker reported a history of either thyroid disease or thyroid medication.

C. Airborne Exposure

The thirty-seven perchlorate participants included fourteen employees in the nominally low perchlorate exposure jobs, 8 employees in the nominally medium perchlorate exposure jobs, and 15 employees in the nominally high perchlorate exposure jobs. All thirty-seven perchlorate participants wore air samplers. Thirty-two wore respirable particle samplers, and twenty-one wore total particle samplers. Seven of the twenty-one azide participants wore air samplers. Six wore respirable particle samplers, and four wore total particle samplers. Table 1 presents the respirable and total airborne perchlorate exposures (mg/day) of the workers, stratified by exposure group. This was calculated from the laboratory report of the amount of perchlorate in the sampling cassettes, the air sampling rate (about 2 liters/min) and duration (about 10 hours), and the assumption of an inhalation rate of 1.2 cubic meters per hour. Data are presented as the arithmetic and geometric means and standard deviations, along with the range, median, and distribution by quartiles. This table demonstrates that airborne perchlorate particle levels are greater in the dusty parts of the perchlorate plant than in the azide plant and that both respirable particle and total particle perchlorate inhalation progressively increase with visible-dust level in the perchlorate plant exposure groups. The exposures of the high exposure perchlorate group are clearly discernible from the other worker groups, being three orders of magnitude greater than those of the azide workers. The minimal exposures of the azide workers may come from contamination from the shared non-production facilities or contamination of their work site. This table also demonstrates that patterns of distribution for respirable and total particles across the exposure groups are similar. Individual measures of respirable and total particle perchlorate were highly correlated with a statistically significant correlation co-efficient ($r = 0.82$; $p << 0.01$), based on 18 paired samples. Respirable particle inhalation accounted for 14% of the total particle inhalation rate (Figure 1).

Particle size-selective sampling conducted in the blender operation (perchlorate exposure group C area) yielded a mass median aerodynamic diameter of the particles of 7.4 μm with a geometric standard deviation of 3.8.

D. Urinary perchlorate levels

Table 2 presents mean urine perchlorate levels (mg/gm creatinine) for the azide plant workers and for each perchlorate exposure group. The data are presented for the pre-shift urine sample, for the post-shift urine sample, and for a post-shift (adjusted) measure. The data are presented as the arithmetic mean and its standard deviation, the range, the median, and the quartiles.

The pre-shift urine was collected prior to the beginning of the work shift, and the post-shift urine was collected at the end of the work shift. The post-shift urine perchlorate measurement reflects two components – (a) the excretion due to the residual in the body of the perchlorate that was present in the body at the time of the pre-shift urine perchlorate measure and (b) the incremental increase in urinary perchlorate due to the excretion of the perchlorate that was inhaled (or ingested) during the work shift.

The post-shift (adjusted) measure is defined to represent the post-shift urinary perchlorate component that reflects perchlorate inhaled during the shift. The body burden represented by the pre-shift urinary perchlorate measure is estimated by pharmacokinetic modeling (assuming first order kinetics and an 8 hour excretory half life) to be reduced by 65% after 12 hours. The post-shift (adjusted) urinary perchlorate estimate was obtained by subtracting 35% of the pre-shift urinary perchlorate measure from the post-shift urinary perchlorate measure as an exposure estimate adjustment. This first-order model follows the equation: $E_i - E_o e^{-kt} = D(1 - e^{-kt})$ where E is the excretion rate of perchlorate at time (o) and time (i) and D is the dose rate from exposure. For a half-life of 8 hours and at time 12 hours, e^{-kt} equals 0.354. The post-shift (adjusted) measure is $E_i - 0.354\ E_o$.

Table 2 demonstrates that workers in the three perchlorate exposure groups have progressively increasing levels of urinary perchlorate. Symmetry about the means and the similarities between the mean and the median in each strata suggest that log transformation of the data is unnecessary.

E. Absorbed dose

The absorbed dose can be calculated for each shift directly from the data presented above in Table 2. From the model equation above, with 12 hour work shifts and an 8 hour half life, the excreted dose (D) follows the equation: $D = k\,[E_i - 0.354\,E_o]/0.646$. The term $[E_i - 0.354\,E_o]$ is the post-shift (adjusted) level in mg perchlorate per gram creatinine. The human adult creatinine excretion rate of 1 mg/min links perchlorate excretion rates in terms of creatinine to rates in terms of time. The percent absorbed that is excreted into the urine is assumed to be 95% as shown by Eichler (1929). The exposure rate is assumed to be relatively constant throughout the work shift and was measured as a time-weighted average exposure. The excreted dose is then 12 hours x 60 min/hr x 0.001 gm/mg x 1 mg creatinine/min x [post-shift adj]/0.646 and the absorbed dose is the excreted dose/0.95 which is expressed in mg/shift. This represents a reasonable estimate of perchlorate absorption over a 12 hour shift (mg/shift) and is calculated independently of the exposure estimates.

F. Thyroid function status

Table 3 presents the mean thyroid function tests for the azide production workers and for each of the perchlorate exposure groups, along with the standard deviations, ranges, medians, and distribution by quartiles. There were no differences in thyroid function tests between workers in the azide and perchlorate plants or between the azide workers and any of the three perchlorate exposure groups. Extreme outlier values due to specific thyroid diseases were excluded from the analysis as indicated in Table 3. Pairwise t-tests were performed between the azide group and each of the three perchlorate exposure groups. As shown in Table 3, none of the comparisons were statistically significant at $\alpha < 0.05$ level. A non-parametric z-test for trend across the ordered groups for each of the six thyroid function tests, using the method developed by Cuzick (1985), found no statistically significant trend (although the trend for TFI was of borderline significance but in the opposite direction than pharmacologically predicted).

Categorical data analyses also were conducted with normal values for T_4, T_3, FTI, and THBR being defined as values at or above the normal lower limit for the laboratory and with normal values of TSH and anti-TPO being defined as values at or below the normal upper limit for the laboratory. There were no significant differences across the exposure groups, between the two plants, or in comparison to the laboratory range of normal. In no case did the proportion of abnormal values for the perchlorate workers exceed that for the azide workers. There were no suggestive trends, either statistically or clinically, for any thyroid function test. Further, no differences in the thyroid function tests were observed in the perchlorate plant workers due to their inhaled and excreted perchlorate levels. These findings show that thyroid function is not altered in workers exposed at the perchlorate levels found in this plant.

G. Thyroid examinations

Clinical examination of the thyroid of all participants revealed no significant thyroid abnormalities in any group. The thyroid glands did not differ in size, texture, or shape between the two groups or across the perchlorate exposure groups. No goiters were detected in any of the workers. A small nodule was detected in a worker in a low perchlorate exposure job. Secondary signs of hypothyroidism and of hyperthyroidism were sought by the thyroid examiner (LEB) and were not observed. There was no evidence of bradycardia, tachycardia, or tremor. Examination of the skin, eyes, and extremities did not reveal signs of thyroid disease.

H. Urinary iodine excretion

The pre-shift urinary iodine values of the azide and perchlorate workers met international standards with 95% or more having a urinary iodine level of 50 ug/l or greater. More than 90% of each group had pre-shift urinary iodine levels greater than 100 ug/l. These data indicate the absence of iodine deficiency in these groups of workers.

The pre-shift urinary iodine values did not differ between the perchlorate workers (mean = 318 μg/l; standard deviation = 164 μg/l) and the azide workers (mean = 344 μg/l; standard deviation = 180 μg/l). Analysis was limited to the forty-nine specimens with pre-shift urinary iodine values less than 800 μg/l, as greater values suggest contamination from extraneous sources. Additionally, the urinary iodine values adjusted for creatinine excretion did not differ between the perchlorate workers (mean = 192 μg/gm; standard deviation = 132 μg/gm) and the azide workers (mean = 211 μg/gm; standard deviation = 120 μg/gm).

Pre-shift and post-shift urinary iodine levels were compared. There was no evidence of increased iodine excretion post-shift compared to pre-shift for either the azide or perchlorate workers, based on those forty-four paired urine samples with values not suggesting contamination (i.e., < 800 ug/l). The pre-shift and post-shift urine iodine levels did not differ for the perchlorate workers [Pre-shift: mean = 294 ug/l, stn dev = 150; Post-shift: mean = 297 ug/l, stn dev = 175] or for the azide workers [Pre-shift: mean = 350 ug/l, stn dev = 189; Post-shift: mean = 325 ug/l, stn dev = 202].

I. Complete Blood Counts

The blood counts showed no difference between the perchlorate workers and the azide workers, either directly or when the perchlorate workers were stratified by exposure groups. The mean of the red cell counts, white blood cell counts (including lymphocytes, neutrophils, monocytes, etc.) and platelet counts revealed no significant differences across the exposure groups (Table 4). The proportion of workers with red cell count, white blood cell counts, or platelet counts below the laboratory normal ranges were similar in all groups. No significant trends were observed in the blood cell data whether examined as continuous variables or as categorical variables. Neither the mean nor the proportion abnormal were found to be significantly different for the azide group or any of the perchloride groups when each of the eighteen cellular parameters were examined. There was no evidence for aplastic anemia, agranulocytosis, or neutropenia. No suggestion of hematotoxicity was seen among the perchlorate-exposed workers or the azide-exposed workers.

J. Other clinical parameters

Serum chemical profiles were conducted on workers. The clinical chemistry results showed no evidence of either renal or hepatic toxicity. The frequency of elevated serum cholesterol levels was greater than 5 % among both the azide and the perchlorate workers. The serum phosphate level was the only chemical variable that showed significantly higher values among the perchlorate workers than among the azide workers. No explanation for this finding is apparent. There were no differences between the groups in the distributions of any of the other twelve chemical profile parameters.

K. Perchlorate excretion rate

Urinary perchlorate levels for two workers in the high-exposure perchlorate group were monitored during three days with measured occupational perchlorate exposure and during the subsequent three days without known perchlorate exposure (Figure 2). The data indicate that the perchlorate body burden, as indicated by urinary perchlorate concentration, increases over the three days of work exposure with generally a decrease between the 12-hour work shifts. Figure 2 graphically illustrates that exposure is the reason for absorption. Figure 3 presents the same data, but as the logarithm of the urinary perchlorate concentration. The elimination of perchlorate after the last definite exposure period appears to follow a 1st order kinetics pattern, which is particularly noted when the urinary perchlorate level is between 0.1 and 10 mg/l (Figure 3). The average perchlorate elimination half-life post-exposure for Employee A was 7.9 hours (excluding the period in day 6 of apparent minimal exposure), and the average perchlorate elimination half-life post-exposure for Employee B was 8.2 hours. The graph of Figure 3 suggests that the excretion half-life may be longer when the urine concentration is greater than 10 mg/l than at lower levels, possibly indicating a re-distribution into an alternative component site, such as the digestive tract.

Discussion

This occupational health study was conducted to determine whether occupational exposure to airborne perchlorate particles and the resultant systemic absorption of perchlorate during the manufacturing process has adversely affected the thyroid status of perchlorate workers. The study revealed no differences in thyroid function tests between perchlorate workers and a comparison group (azide workers). No differences in thyroid function test results were found among the workers across the four exposure groups.

The perchlorate characteristics of these four groups can be described both in terms of airborne perchlorate exposure and of perchlorate absorption as determined from urinary perchlorate excretion. Based on industrial hygiene airborne measurements, these groups are found to have had exposures with group arithmetic mean exposures ranging from 0.01 to 60 mg perchlorate per day, group median exposures ranging from 0.01 to 45 mg/day, and group geometric mean exposures ranging from 0.0l to 30 mg perchlorate per day. Different analysts may consider different exposure metrics to best summarize the airborne exposure. Based on urinary perchlorate excretion data, these groups are found to have had absorbed dosages with means ranging from 0.9 to 34 mg/day and medians ranging from 0.6 to 33 mg/day. The dosage data distribute symetrically about the means and the means are almost identical to the medians. Therefore, geometric means would provide no additional information. These data demonstrate no adverse effect on thyroid function at perchlorate absorptions of 0.01 to 34 mg/day.

No occupational thyroid disease was found among these workers. The perchlorate exposures in this plant were not found to be associated with thyroid abnormalities. Further, there was no evidence of differences between the groups with respect to renal, hepatic, or hematological parameters. Thus, there is no evidence that perchlorate affects the thyroid or those other three systems at these absorption levels, a daily absorption of 34 mg/day.

This study is the first to measure and assess urinary perchlorate concentrations in workers exposed to perchlorate particles. This study includes two measures of perchlorate exposure (total and respirable particles) and one measure of perchlorate absorption (urinary perchlorate). The two measures of perchlorate exposure have been found to correlate very strongly (Figure 1). Data analysis has been conducted to examine the relationship between airborne particle perchlorate (both respirable and total) and perchlorate absorption.

Figure 4 demonstrates the statistically significant association (correlation) between airborne respirable particle perchlorate exposure and perchlorate absorption. The slope of the association (b = 2.06) is greater than one, indicating that although the rise in respirable particle exposure significantly tracts the rise in absorption, the increase in absorption is twice as great as the increase in this exposure metric. Thus, the respirable particle perchlorate exposure is insufficient to account by itself for the rise in the perchlorate absorption.

Figure 5 demonstrates the statistically significant association (correlation) between airborne total particle perchlorate exposure and perchlorate absorption. In this case, the slope of the association (b = 0.31) is less than one, indicating that the increase in total particle perchlorate exposure is sufficient to account for the increase in the perchlorate absorption. This analysis suggests that an absorption co-efficient of 31% could describe the association between total particle perchlorate exposure and perchlorate absorption. Inspection of Figure 5 suggests that at lower total particle perchlorate exposures (i.e., 0 to 50 mg/day) other factors, such as hand-to-mouth ingestion, may make a major contribution to the total perchlorate exposure and absorption.

The airborne total particle perchlorate, with its high aqueous solubility, appears to be readily absorbed and appears generally to be the source of the excreted perchlorate. The design of pre- and post-shift urine collections and perchlorate assessments and measurements of both respirable particle and total particle inhalation exposure to perchlorate throughout the shift has revealed that total particle (as well as respirable particle) perchlorate inhalation exposure leads to systemic absorption of perchlorate and to its urinary excretion. The lower respiratory tract is the primary site for respirable particle perchlorate to be absorbed. Whether the total particle perchlorate is also absorbed in the upper respiratory tract and is carried by mucous into the gastrointestinal tract, or it enters the gastrointestinal tract by direct contact, is not important to the post-absorption pharmacology. The perchlorate absorbed into the blood stream (whether from the respiratory or gastrointestinal tract) are equivalent since perchlorate excretion in the urine and pharmacological effects on the thyroid are both dependent upon absorption into the blood stream.

The data from the workers in this study contributes to the developing literature on perchlorate excretion rates in humans. The excretion half-lives of 7.9 hours and 8.2 hours in the workers observed for three days post-exposure is quite consistent with the 6-8 hours reported by Eichler in 1929 and the 5-9 hours reported by Durand in 1938. There is a quiet pleasure in observing that one's work replicates data published sixty to seventy years earlier, though the total number of subjects published is now only four.

The Eichler (1929) exposure was to a single oral dose of 1 or 2 grams. The Durand (1938) exposure was to a single oral dose of 0.8 grams. The two workers in this study had been working in perchlorate production area C during the prior work period and can be assumed to have had the equivalent of a 34 mg oral dosage over a twelve hour period. Since these workers are regularly in this employment, this exposure can be described as chronic or sub-chronic exposure at a moderate dosage (greater than environmental and less than pharmaceutical). Although there is a suggestion within our data that some other physiological processes may be occurring at higher exposure levels, these data do indicate that 8-hours is a reasonable estimate of the perchlorate excretion rate in humans. This 8-hr half-life has been consistent down to the limit of detection in the urine.

This study has provided an insight into the absorption and excretion of perchlorate among workers exposed to airborne perchlorate particles. It has also shown that these workers do not demonstrate any adverse effect on their thyroids at these occupational exposure levels at this perchlorate-manufacturing plant. This study also confirms the findings of Gibbs et al. that demonstrated the absence of an adverse effect on thyroid function among perchlorate-manufacturing employees at a different plant.[16] Gibbs demonstrated the absence of an effect on thyroid function both in examining across the work shift acutely and across the working life cumulative exposure chronically.[16] Gibbs et al. also demonstrated the absence of an effect on kidney, liver, or bone marrow function across the working life.[16] The present study has added observations of individual respiratory perchlorate particle exposures and subsequent urinary perchlorate measurements to the exposure measurements of Gibbs et al.[16] It has also added serum T_3 and anti-TPO antibodies, and a clinical thyroid examination to their assessment of thyroid function outcome measures. This study has also reported the absence of an effect on the liver, kidney, or blood cells at a range of perchlorate exposure and absorption rates. Both studies have demonstrated that occupational exposures to perchlorate have not been hazardous to the thyroid health status of the workers studied at these plants or to the other examined organ systems.

Occupational health studies serve to advise workers, physicians, and managers on the safe limits of exposure. That is their primary function and their purpose of design. Such studies may also be helpful to toxicologists and other health scientists who desire clarification of mechanisms and parameters concerning perchlorate exposure, absorption, toxicity, and excretion. Additionally, such studies are useful to environmental health specialists who must consider the risks associated with low-level exposures to perchlorate, either through inhalation or ingestion. The US Environmental Protection Agency and a number of state health departments are currently attempting to assess the potential magnitude of risk associated with various levels of perchlorate contamination of drinking water. Studies such as this provide useful information for the assessment of such risks.

Current levels of perchlorate detected in drinking waters of Southern California and Southern Nevada are in the range of 5-8 ppb (μg/l) and up to 15 ppb, respectively. The assumed ingestion of two liters per day would yield an ingestion exposure and absorption of up to 30 μg perchlorate per day for an adult. This rate is about one to two orders of magnitude lower than that of the azide worker control group in this study. It is about three orders of magnitude lower than that of the perchlorate group C workers who showed no adverse effect on their thyroid health status with recurrent occupational absorbed exposures of about 34 mg perchlorate per day.

Two issues of perchlorate toxicity have arisen, hematotoxicity in adults and congenital hypothyroidism in the newborn. Cases of hematotoxicity associated with perchlorate exposure have generally been reported for Grave's disease patients being treated therapeutically with exposures of 1,000 mg per day or greater, occasionally at 600 mg per day, and only once in a patient at 450 mg per day (who had previously shown a toxic reaction at 800 mg per day).[17] No hematotoxicity was seen among the workers in this study and, in particular, not at 34 mg per day. The case report literature suggests that reported hematotoxicity findings in Grave's disease patients may occur at exposures at least one to two orders of magnitude greater than the occupational exposures and at least four to six orders of magnitude greater than the environmental exposures from the Southern California and Nevada waters.

Prior to 1960, perchlorate was commonly used to treat women with hyperthyroidism during pregnancy. Crooks and Wayne reported in Lancet that they had "treated 12 pregnant thyrotoxic patients with potassium perchlorate (600 mg/day or 1000 mg/day) and in each have achieved satisfactory control of the disease.[18] One of the infants had a very slight enlargement of the thyroid that disappeared within 6 weeks. The remainder showed no abnormality of any kind." This is the only published report of perchlorate and the neonatal thyroid. Additionally, the California Department of Health Services (1997) has a preliminary health review for a Superfund site in Sacremento, California where perchlorate is a contaminant of concern.[19] They found no increase in congenital hypothyroidism in the zip codes of interest. Similarly, Doemland and Lamm reported that the counties in Southern California and Nevada with perchlorate-contaminated drinking water had no more cases of congenital hypothyroidism than would be expected, based on state rates for 1996 and 1997.[20] Thus, only one case of transient goiter in a newborn has been reported for those whose mother had therapeutic exposures and two research groups looking at different geographic areas found no evidence of an increased risk of congenital hypothyroidism with environmental exposures.

In conclusion, this study has (1) found no evidence of an adverse effect of perchlorate exposure on thyroid health status among perchlorate workers, (2) demonstrated that airborne perchlorate is absorbed and excreted by perchlorate workers, (3) indicated that the exposure to perchlorate particles larger than respirable size is likely to account for the magnitude of perchlorate excretion, (4) provided an estimate for the urinary excretion half-life of perchlorate in perchlorate workers, and (5) developed information that may be useful in assessment of human risk from environmental exposure to perchlorate. The results of the present study do not support the hypothesis that chronic exposure to perchlorates at the levels encountered in this study has an effect on thyroid function. There also is no evidence to support the hypothesis that perchlorate has an effect on the hematopoeitic system even at these occupational doses. The findings in this study demonstrate a "no adverse effect level" on thyroid function and hematotoxicity in a worker population of 34 mg perchlorate per day for humans.

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References

1. Buchinger W, Lorenz-Wawscinek O, Semlitch G et al. Thyrotropin and Thyroglobulin as an Index of Optimal Iodine Intake: Correlation with Iodine Excretion of 39,913 Euthyroid Patients (in Austria). *Thyroid.* 1997;7(4): 593-597.

2. Wolff J. Perchlorate and the thyroid gland. *Pharmacol Rev.* 1998;50(1):89-105.

3. Eichler O. On the pharmacology of perchlorate (in German). *Arch Exp Path Pharm.* 1929;144:251-260.

4. Durand MJ. Recherches sur l'elimination des perchlorates, sur leur repartition dans les organes et sur leur toxicite. *Bull Soc Chim Biol.* 1988; 20: 428-435.

5. Stanbury JB and Wyngaarden JB. Effect of perchlorate on the human thyroid gland. *Metab Clin Exp.* 1952;1:533-539.

6. Wenzel KW and Lente JR. Similar effects of thionamide drugs and perchlorate on thyroid-stimulating immunoglobulins in Graves' Disease: Evidence against an immunosuppressive action on thionamide drugs. *J Clin Endocrinol Metab.* 1984; 58(1):62-69.

7. Martino E, Aghini-Lombardi F, Mariotti S, et al. Treatment of amiodarone associated thyrotoxicosis by simultaneous administration of potassium perchlorate and methimazideideole. *J Endocrin Invest.* 1986; 9:201-207.

8. Martino E, Mariotti S, Aghini-Lombardi F, et al. Short-term administration of potassium perchlorate restores euthyroidism in amiodarone iodine-induced hypothyroidism. *J Clin Endocrin Metab.* 1986; 63(5):1233-1236.

9. Reichert LJ and deRooy HAM. Treatment of amiodarone induced hyperthyroidism with potassium perchlorate and methimazideideole during amiodarone treatment. *BMJ.* June 10,1989; 298:1547-1548.

10. Trip MD, Duren DR, and Wiersinga WM. Two cases of amiodarone-induced thyrotoxicosis successfully treated with a short course of antithyroid drugs while amiodarone was continued. *Br Heart J.* 1994;72: 266-268.

11. Burgi H, Benguerel M, Knopp J, Kohler H, and Studer H. Influence of perchlorate on the secretion on non-thyroxine iodine by the normal human thyroid gland. *Eur J Clin Invest*, 1974; 4: 65-69.

12. Brabant G, Bergmann P, Kirsch CM, Kohrle J, Hesch RD, Von Zur Muhlen A. Early adaptation of thyrotropin and thyroglobulin secretion to experimentally decreased iodine supply in man. *Metabolism*, 1992; 4: 1093-1096.

13. Benotti J, Benotti N, Pino S, and Gardyna H. Determination of total iodine in urine, stools, diets, and tissue. *Clin Chem,* 1965 Oct 1; 11(10):932-936.

14. Richman KW, Howearth G, and Lamm SH. Quantitative determination of perchlorate ion concentrations in urine. Abstract at the 1999 AIHCE, Toronto.

15. Cuzick, J. 1985. A Wilcoxon-type test for trend. Statistics in Medicine 4:87-90.

16. Gibbs JP, Ahmad R, Crump KS, Houck DH, Leveille TS, Findley JE, Francis M.: Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function *J Occup Environ Health,* 1998 Dec; 40(12):1072-1082.

17. Krevans JR, Asper SP, Jr., Rienhoff WF, Jr. Fatal aplastic anemia following use of potassium perchlorate in thyrotoxicosis. *JAMA*, 1962; 181: 162-164.

18. California Department of Health Services. Preliminary health review in Rancho Cordova, Sacramento County, California. 1997.

19. Crooks J and Wayne EJ. Potassium perchlorate, methylthiouracil, and carbimazole in the treatment of thyrotoxicosis. *Lancet*, 1960 Feb; 1: 401- 404.

20. Doemland ML and Lamm SH. Perchlorate in drinking water and risk of congenital hypothyroidism. J Occ·Environ Med, 1999 (under review).

Table 1. Descriptive statistics of respirable and total airborne perchlorate levels (mg/day) by plant and exposure groups

Groups	N	Ar-Mean	Ar-STD	Geo-Mean	Geo-STD	Min	P25	Median	P75	Max
					Respirable (mg/day)					
Azide	6	0.021	0.014	0.017	1.925	0.009	0.010	0.014	0.038	0.039
Perchlorate A	11	0.091	0.095	0.057	3.019	0.006	0.040	0.067	0.083	0.331
Perchlorate B	7	0.601	0.671	0.255	5.196	0.031	0.031	0.374	1.522	1.575
Perchlorate C	14	8.591	9.386	5.414	2.740	0.957	2.643	5.040	10.160	35.852
					Total (mg/day)					
Azide	4	0.014	0.012	0.011	2.482	0.004	0.006	0.012	0.023	0.030
Perchlorate A	6	0.337	0.187	0.288	1.921	0.107	0.168	0.330	0.487	0.602
Perchlorate B	2	6.567	7.139	4.200	---	1.519	1.519	6.567	11.615	11.615
Perchlorate C	12	59.378	53.605	28.674	5.101	1.036	11.772	44.890	103.749	166.996

Legend:

N = number; Ar-mean = arithmetic mean (average); Ar-STD = standard deviation of the arithmetic mean; Geo-mean = geometric mean (logarithmic mean); Geo-STD = standard deviation of the geometric mean; Min = minimum value; P25 = 25th percentile value; median = 50th percentile value; P75 = 75th percentile value; and Max = maximum value.

Table 2. Descriptive statistics of creatinine-adjusted urine perchlorate levels (mg/gm) by plant and exposure and adsorbed dose (mg/shift)

Groups	N	Mean	Std. Dev.	Min	P25	Median	P75	Max
				Pre-shift				
Azide	21	1.31	1.55	0.23	0.39	0.84	1.36	6.37
Perchlorate A	14	2.05	2.42	0.42	0.59	1.09	1.50	8.02
Perchlorate B	8	5.98	5.70	0.64	1.12	4.87	9.91	15.41
Perchlorate C	14	11.30	9.93	0.53	1.09	14.89	17.61	30.22
				Post-shift				
Azide	21	1.19	1.16	0.16	0.52	0.81	1.52	4.97
Perchlorate A	14	4.07	2.15	0.46	2.38	3.53	5.71	8.24
Perchlorate B	8	11.27	7.59	3.63	4.93	9.79	16.45	24.22
Perchlorate C	15	32.22	13.14	11.16	27.81	33.09	38.89	64.38
				Post-Shift (adjusted)				
Azide	21	0.75	1.00	-0.85	0.14	0.53	0.78	3.62
Perchlorate A	14	3.39	2.29	0.32	1.69	2.87	5.02	8.04
Perchlorate B	8	9.28	7.41	2.67	3.13	7.16	13.32	23.98
Perchlorate C	14	28.66	12.38	10.80	21.04	28.43	33.70	58.51
				Absorbed Dosage (mg/shift)				
Azide	21	0.88	1.17	-1.00	0.16	0.62	0.92	4.25
Perchlorate A	14	3.98	2.69	0.38	1.98	3.37	5.89	9.43
Perchlorate B	8	10.89	8.69	3.13	3.67	8.40	15.63	28.13
Perchlorate C	14	33.62	14.52	12.67	24.68	33.35	39.54	68.65

Table 3. Descriptive statistics of thyroid function parameters by plant and exposure groups

Groups	N	Mean	Std. Dev.	Min	P25	Median	P75	Max	P-value*
				T4 (5 – 11 µg/dl)**					
Azide	21	6.73	1.479	4.60	5.40	6.80	7.40	9.90	—
Perchlorate A	13	7.13	1.583	4.00	6.40	6.90	7.80	10.60	0.46
Perchlorate B	8	7.34	1.115	5.40	6.70	7.50	8.00	8.90	0.31
Perchlorate C	15	7.03	1.301	4.40	6.00	7.30	8.10	8.60	0.54
				T3 (87 – 178 ng/dl)					
Azide	21	142.52	17.543	113.00	129.00	143.00	156.00	169.00	—
Perchlorate A	13	148.38	25.178	96.00	145.00	159.00	166.00	174.00	0.43
Perchlorate B	8	152.13	23.234	120.00	134.00	148.00	176.00	181.00	0.24
Perchlorate C	15	152.13	20.368	108.00	141.00	150.00	165.00	192.00	0.14
				TSH (0.45 - 4.5 µU/ml)					
Azide	21	3.14	1.870	0.67	2.00	2.80	4.10	8.40	—
Perchlorate A	12	2.68	1.143	1.20	1.70	2.50	3.75	4.50+	0.45
Perchlorate B	8	2.41	1.271	0.75	1.45	2.15	3.50	4.30	0.32
Perchlorate C	15	3.33	2.338	0.65	1.50	2.80	4.20	8.20	0.80
				FTI (5.0 - 11.0)					
Azide	21	6.05	1.248	4.40	5.30	6.00	6.70	9.60	—
Perchlorate A	13	6.33	1.435	3.20	5.80	6.40	6.90	9.40	0.55
Perchlorate B	8	6.56	0.847	5.10	6.25	6.50	6.90	8.10	0.29
Perchlorate C	15	6.56	1.022	4.40	5.60	6.90	7.20	8.20	0.20
				THBR (0.85 – 1.10)					
Azide	21	0.90	0.071	0.80	0.84	0.91	0.95	1.08	—
Perchlorate A	13	0.89	0.064	0.79	0.83	0.89	0.93	0.99	0.47
Perchlorate B	8	0.90	0.069	0.79	0.85	0.90	0.96	0.99	0.81
Perchlorate C	15	0.94	0.094	0.78	0.87	0.95	1.01	1.09	0.19

	Anti-TPO (< 20 IU/ml)								
Azide	21	9.28	11.274	5.00	5.00	5.00	6.30	53.20	—
Perchlorate A	13	9.26	10.611	5.00	5.00	5.00	5.00	42.70	0.98
Perchlorate B	8	11.38	9.524	5.00	5.00	5.00	18.20	29.60++	0.65
Perchlorate C	14	8.63	6.797	5.00	5.00	5.00	9.50	29.60	0.85

* All t-tests were performed assuming equal variances based on Bartlett's test for equal variances (at α level of 0.05).

** Values in parentheses represent the laboratory's normal range for the assay.

\+ One extreme outlier (TSH = 38 µU/ml) was excluded. This worker was diagnosed with Graves' Disease nine years before employment and is insufficiently treated for his hypothyroidism that developed following ^{131}I therapy.

++ One extreme outlier (Anti-TPO = 709 IU/ml) was excluded. This worker has euthyroid Hashimoto's thyroiditis.

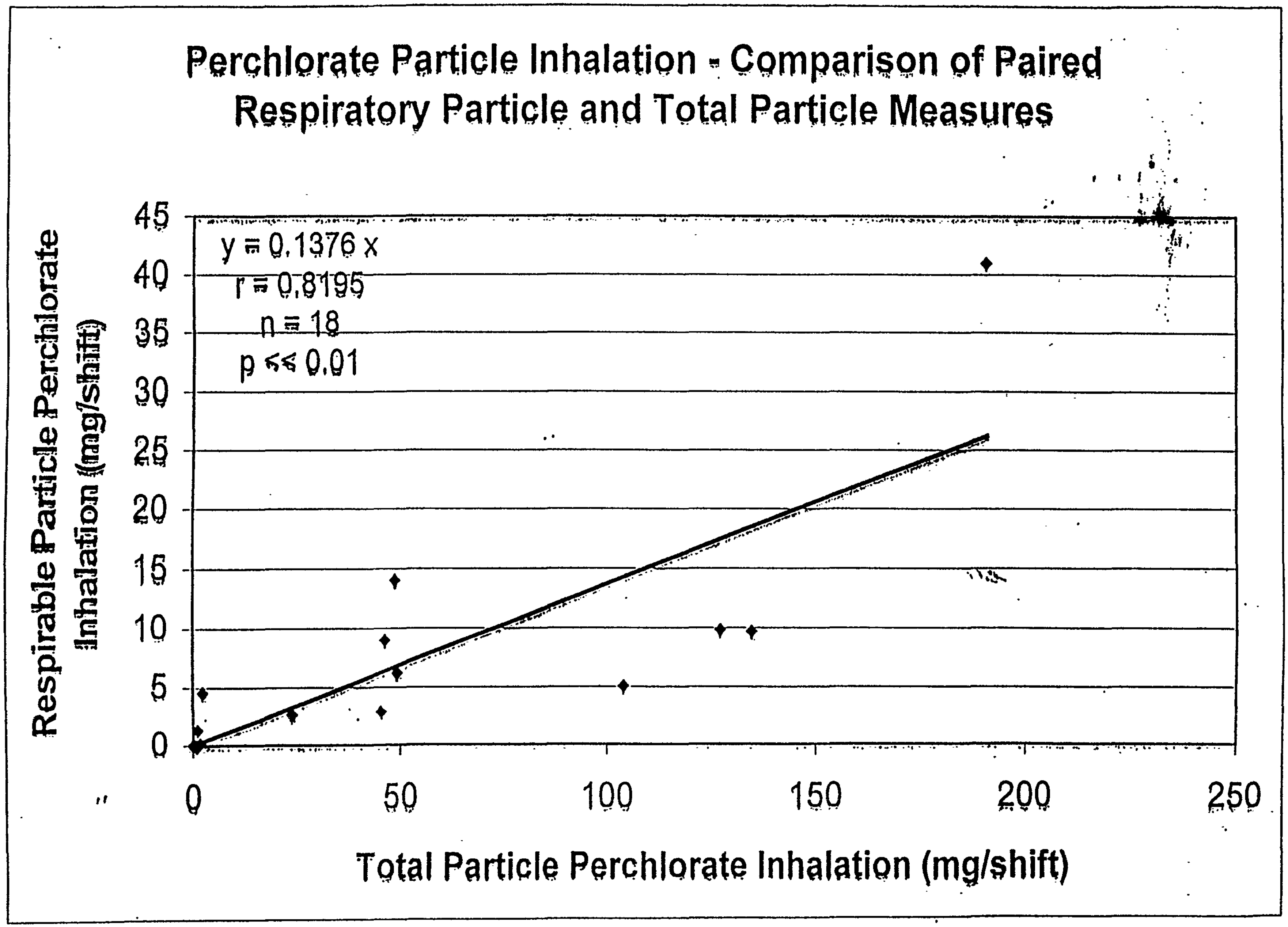
Table 4. Descriptive statistics of blood cell counts by plants and exposure groups

Groups	N	Mean	Std. Dev.	Min	P25	Med	P75	Max	P-value
				Red Blood Cells (4.40 – 5.80 x 10^3/nl)**					
Azide	21	5.16	0.333	4.30	5.00	5.20	5.40	5.73	—
Perchlorate A	14	5.06	0.500	4.10	4.90	5.22	5.30	5.70	0.48
Perchlorate B	8	5.11	0.233	4.74	4.95	5.14	5.28	5.40	0.66
Perchlorate C	15	5.37	0.367	4.40	5.00	5.50	5.60	5.76	0.10
				White Blood Cells (3.6 – 10.6 cells/nl)					
Azide	21	8.62	5.608	4.90	6.81	7.60	8.30	32.60	—
Perchlorate A	14	7.67	1.380	5.50	7.20	7.30	7.90	11.00	0.47*
Perchlorate B	8	7.83	2.631	4.40	5.45	7.85	9.90	11.80	0.61*
Perchlorate C	15	7.99	1.554	5.90	6.50	7.80	8.70	11.80	0.63*
				Neutrophiles (1.8 – 8.0 cells/nl)					
Azide	21	4.96	5.222	1.80	3.50	3.60	4.40	27.50	—
Perchlorate A	14	4.41	0.796	2.90	4.10	4.65	4.80	5.50	0.64*
Perchlorate B	8	4.20	1.546	2.30	3.00	4.40	4.65	7.20	0.55*
Perchlorate C	15	4.30	1.214	2.20	3.40	4.30	4.70	7.70	0.58*
				Lymphocytes (1.2 – 3.4 cells/nl)					
Azide	21	2.66	0.691	1.30	2.30	2.60	2.80	4.10	—
Perchlorate A	14	2.39	0.659	1.60	1.90	2.20	2.60	4.30	0.27
Perchlorate B	8	2.83	1.516	1.50	1.75	2.30	3.55	5.90	0.77*
Perchlorate C	15	2.81	0.822	1.10	2.40	2.80	3.10	4.40	0.56
				Platelets (140-440 platelets/nl)					
Azide	21	233.00	40.107	149.00	206.00	230.00	268.00	304.00	—
Perchlorate A	14	235.07	47.798	159.00	204.00	245.00	270.00	317.00	0.89
Perchlorate B	8	221.88	61.989	144.00	182.50	213.00	246.00	348.00	0.57
Perchlorate C	15	230.53	57.679	127.00	193.00	222.00	258.00	343.00	0.88

* t-tests were performed assuming unequal variances based on Bartlett's test for equal variances (at α level of 0.05).

** Values in parentheses represent the laboratory's normal range.

Figure 1



Perchlorate Particle Inhalation - Comparison of Paired Respiratory Particle and Total Particle Measures
y = 0.1376 x
r = 0.8195
n = 18
p << 0.01
Respirable Particle Perchlorate Inhalation (mg/shift)
Total Particle Perchlorate Inhalation (mg/shift)
0
5
10
15
20
25
30
35
40
45
0
50
100
150
200
250

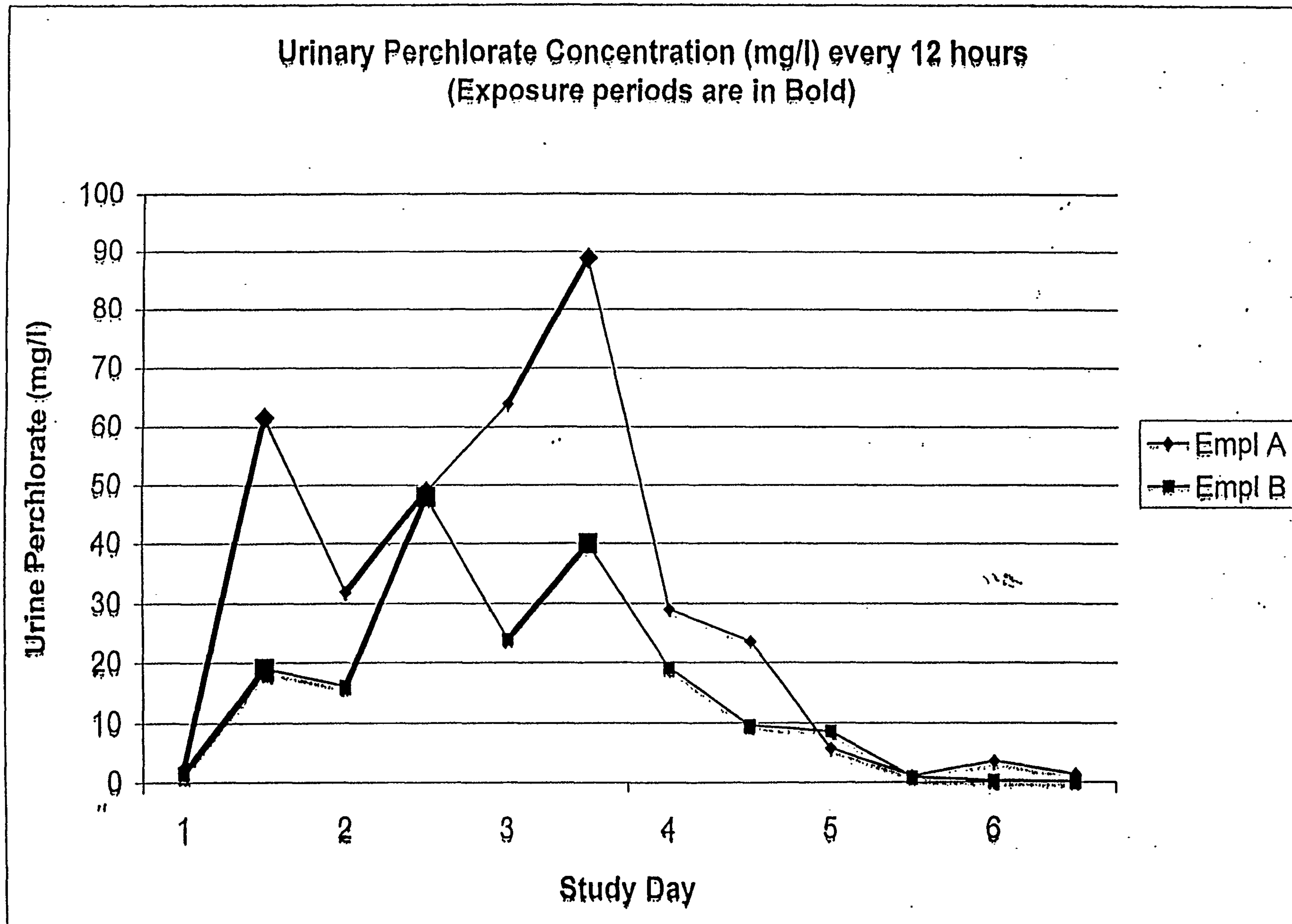
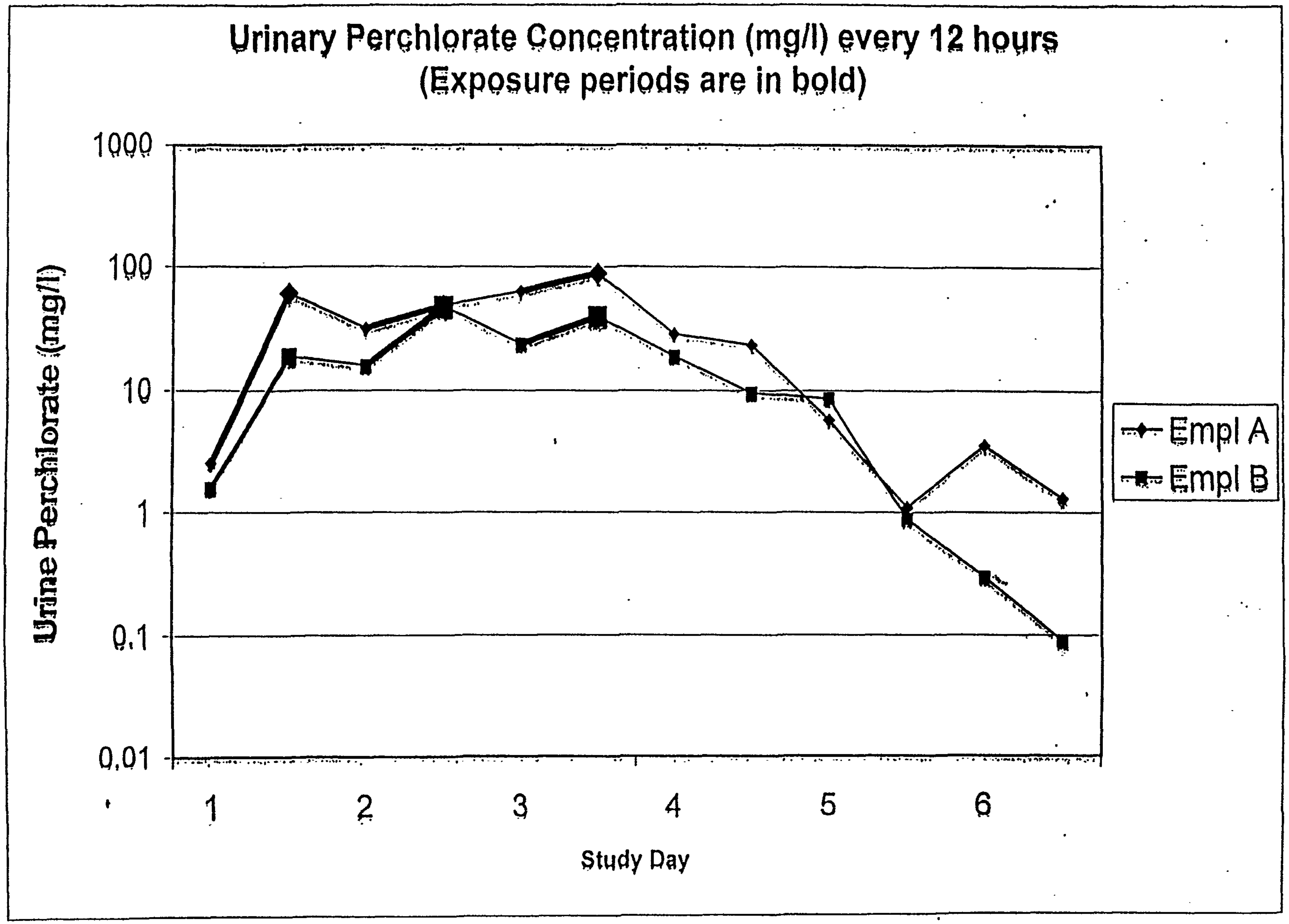
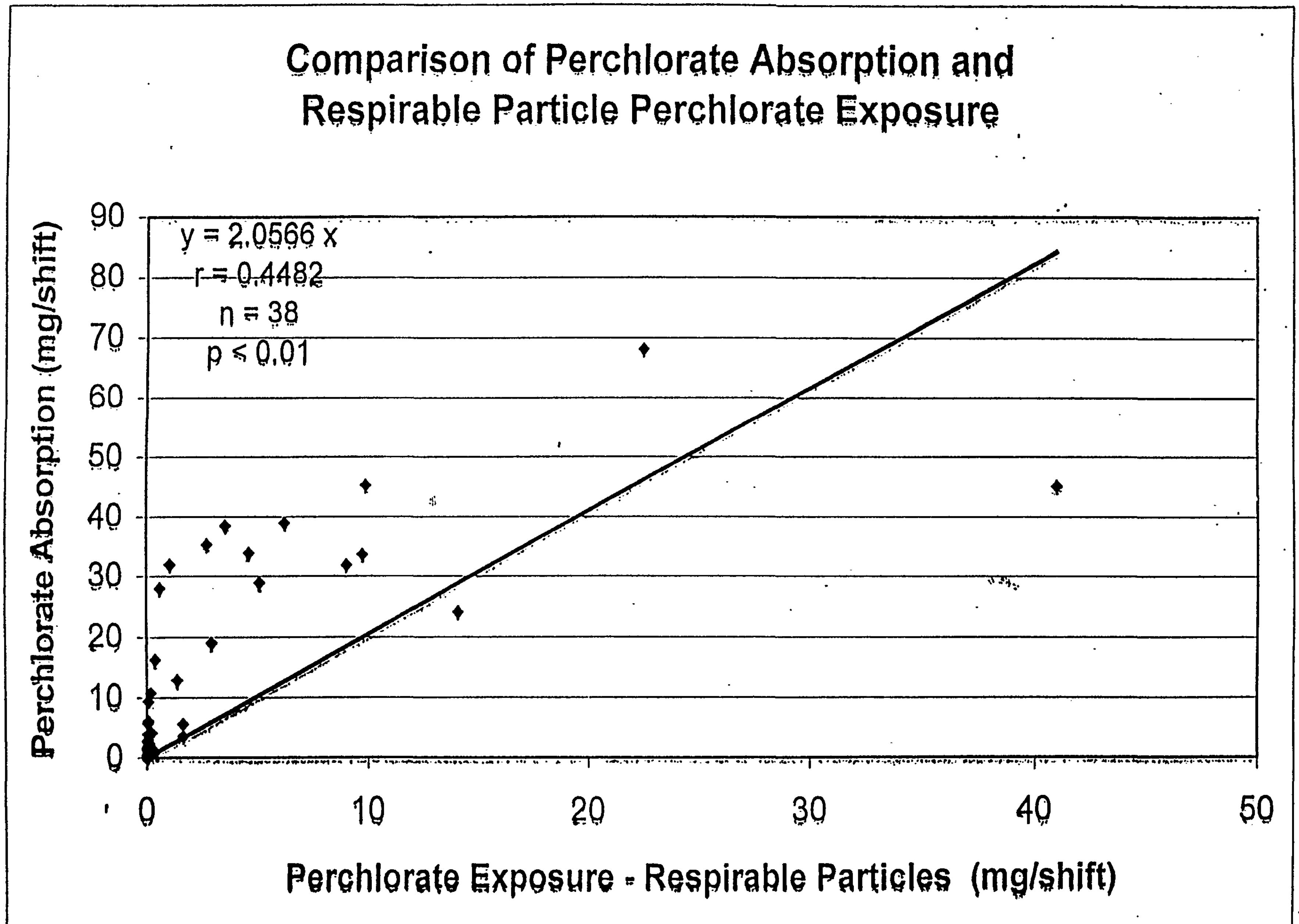
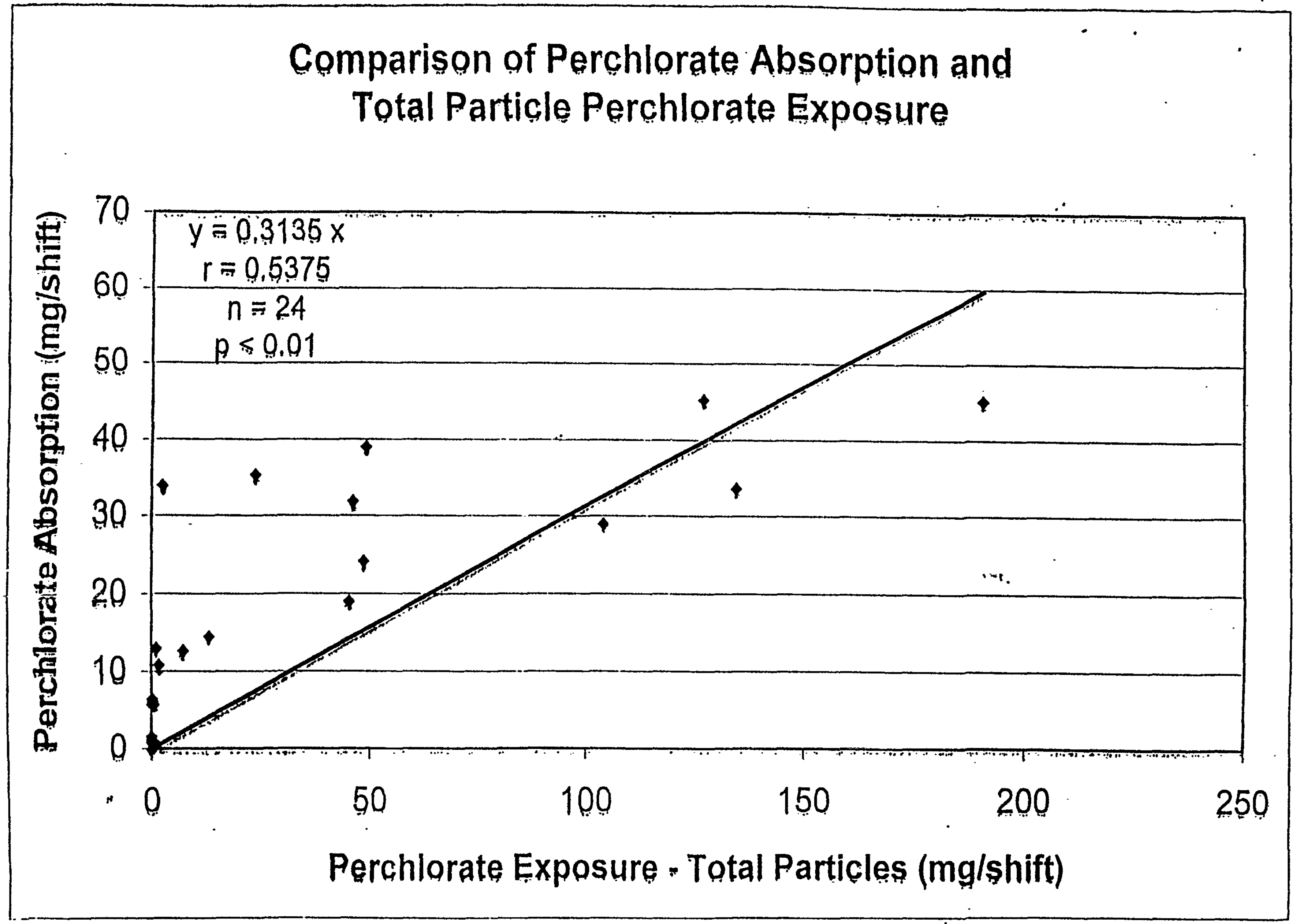

Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in Bold)
Urine Perchlorate (mg/l)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Study Day
Empl A
Empl B

Figure 3



Urinary Perchlorate Concentration (mg/l) every 12 hours
(Exposure periods are in bold)
1000
100
10
1
0.1
0.01
Urine Perchlorate (mg/l)
1
2
3
4
5
6
Study Day
Empl A
Empl B


Comparison of Perchlorate Absorption and
Respirable Particle Perchlorate Exposure
y = 2.0566 x
r = 0.4482
n = 38
p < 0.01
Perchlorate Absorption (mg/shift)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
Perchlorate Exposure - Respirable Particles (mg/shift)


Comparison of Perchlorate Absorption and
Total Particle Perchlorate Exposure
y = 0.3135 x
r = 0.5375
n = 24
p < 0.01
Perchlorate Absorption (mg/shift)
0
10
20
30
40
50
60
70
0
50
100
150
200
250
Perchlorate Exposure - Total Particles (mg/shift)